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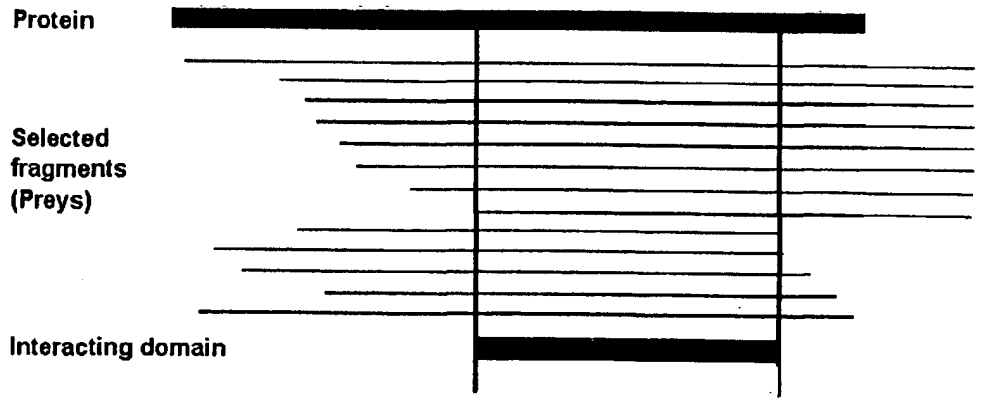

(57) Abstract: The present invention relates to protein-protein interactions involving Breast Cancer Membrane Proteins-7 and -11 (BCMP-7 and BCMP-11). More specifically, the present invention relates to complexes of the interacting proteins, antibodies to the complexes, selected interaction domains of the protein-protein interactions, methods for screening for agents which modulate the protein-protein interactions and pharmaceutical compositions comprising active agents that are capable of modulating the protein-protein interactions.

WO 03/075014 A2

PROTEIN-PROTEIN INTERACTIONS IN HUMAN TUMOURS

The present invention relates to protein-protein interactions involving Breast Cancer Membrane Proteins-7 and -11 (BCMP-7 and BCMP-11). More specifically, the present invention relates to complexes of the interacting proteins, antibodies to the complexes, selected interaction domains of the protein-protein interactions, methods for screening for agents which modulate the protein-protein interactions and pharmaceutical compositions comprising active agents that are capable of modulating the protein-protein interactions.

BCMP-7 was isolated from T-47D cell membranes, purified by 1D gel electrophoresis and characterised by mass spectrometry before being cloned. Expression of BCMP-7 mRNA in human tissues was found to be elevated in some breast cancer cell lines and prostate cancer cell lines, by comparison to the corresponding normal tissues (WO 01/63290). Besides, BCMP-7 was shown to be over-expressed in clinical samples from breast cancer patients when compared to matched normal tissues (see WO 01/63290; Fletcher *et al.*, 2003, Brit. J. Cancer, 88:579-585) and in pancreatic tumour tissue (Fletcher *et al.*, 2003, Brit. J. Cancer, 88:579-585). BCMP-7 expression was shown to be coincident with expression of the estrogen receptor, and BCMP-7 mRNA levels in MCF7 cells increased when the cells were treated with estradiol (Thompson & Weigel, 1998, Biochem. Biophys. Res. Commun. 251, 111-116).

BCMP-11 is a protein sharing 71% identity with BCMP-7. The sequence similarity at the N-termini is relatively low. BCMP-11, like BCMP-7, is predicted to be a secreted protein with an N-terminal signal sequence.

BCMP-11 was isolated from T-47D cell membranes, purified by 1D gel electrophoresis and characterised by mass spectrometry before being cloned. The BCMP-11 gene was shown to be localised to chromosomal region 7p21, in the same contig as BCMP-7 (see WO 01/63289). Expression of BCMP-11 mRNA in human tissues was shown to be elevated in clinical samples from breast cancer patients when compared to matched normal tissues (see WO 01/63289; Fletcher *et al.*, 2003, Brit. J. Cancer, 88:579-585) and in pancreatic tumour tissue (Fletcher *et al.*, 2003, Brit. J. Cancer, 88:579-585). Expression of BCMP-11 was shown to be correlated with a positive estrogen receptor status, as well as with a negative EGF receptor status (see WO 01/63289).

Most biological processes involve specific protein-protein interactions. Protein-protein interactions which may be transient or otherwise, enable two or more proteins or subunits to associate. A large number of non-covalent bonds form between the proteins when two protein surfaces are precisely matched. These bonds account for the specificity of recognition. Thus protein-protein interactions are essential components of for example, DNA replication, transcription, translation, splicing, secretion, cell cycle control, signal transduction and intermediary metabolism. Protein-protein interactions can have a number of measurable effects: they can alter kinetic properties of proteins, they are a common mechanism for allowing substrate channeling; they can result in the formation of new binding sites, they can alter the activity of a protein and protein-protein interactions can alter

the specificity of a protein for its substrate (Phizicky and Fields, 1995, Microbiological Reviews, 59: 94-123; Pawson and Nash, 2000, Gene Dev. 14: 1027-1047).

General methodologies to identify interacting proteins or to study these interactions have been developed. Among these methods is the two-hybrid system originally developed by Fields and co-workers and described, for example, in US 5,283,173, 5,468,614 and 5,667,973

A summary of the available methodologies for detecting protein-protein interactions is described by Vidal & Legrain (1999, Nucl. Acids Res. 27:919-929) and Legrain & Selig (2000, FEBS Letts. 480:32-36). However, these conventional approaches and especially the commonly used two-hybrid methods have their drawbacks. For example, it is known in the art that, more often than not, false positives and false negatives exist in the screening method. In fact, a doctrine has been developed in this field for interpreting the results and in common practice an additional technique such as co-immunoprecipitation or gradient sedimentation of the putative interactors from the appropriate cell or tissue type are generally performed. The methods used for interpreting the results are described by Brent and Finley, Jr. (1997, Ann. Rev. Genet. 31:663-704). Thus, the data interpretation can be unreliable using such systems.

One method, which overcomes the difficulties encountered with the methods in the prior art, is described in WO 99/42612. Using this method the presence of false negatives and/or false positives can be minimised. Another method which can be used *in lieu* of yeast, uses *Escherichia coli* in a bacterial two-hybrid system. This method encompasses a similar principle to that described above for yeast, but does not involve mating for characterizing the prey polynucleotide.

The implementation of screening programmes for the early detection of breast cancer, and the advent of anti-cancer treatments such as chemotherapy, radiotherapy and anti-oestrogen therapies, to augment surgical resection have improved the survival of breast cancer patients. However, even breast tumours with good prognoses such as Oestrogen Receptor positive ductal carcinoma *in situ* (DCIS) become refractory to such treatments as the cancer cells develop resistance to chemotherapy drugs or lose their hormone sensitivity, leading to recurrent or metastatic disease that is often incurable. Thus, further characterisation of the molecular pathology of breast cancer including the identification of other targets, remains a key requirement in the development of better treatments.

The present invention addresses this problem and provides previously unidentified interacting partners for BCMP-7 and BCMP-11 which are of use in the development of more effective and better targeted therapeutic treatments for cancer.

Accordingly, there is provided an isolated or recombinant protein complex comprising:

(a) an interacting polypeptide which:

(1) comprises or consists of the amino acid sequence of SEQ ID NOS:14, 17 or 20; or

(2) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence of SEQ ID NOS: 14, 17 or 20, which retains the interaction domain of the interacting polypeptide;

and a BCMP-11 polypeptide which:

(i) comprises or consists of the amino acid sequence of SEQ ID NO:1; or

(ii) is a derivative having one or more amino acid substitutions, modifications, deletions or insertions relative to the amino acid sequence of SEQ ID NO: 1 which retains the ability to interact with the interacting polypeptide;

or

(b) an interacting polypeptide which:

(1) comprises or consists of the amino acid sequence of SEQ ID NOS:13, 15, 16, 18, 19 or 35; or

(2) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence of SEQ ID NOS:13, 15, 16, 18, 19 or 35, which retains the interaction domain of the interacting polypeptide;

and a BCMP-7 polypeptide which:

(i) comprises or consists of the amino acid sequence of SEQ ID NO:2; or

(ii) is a derivative having one or more amino acid substitutions, modifications, deletions or insertions relative to the amino acid sequence of SEQ ID NO:2 which retains the ability to interact with the interacting polypeptide.

As will be apparent to one skilled in the art, an interacting polypeptide can consist of any of the selected interaction domain sequences of SEQ ID NOS:13 to 20, or 35, or may comprise a larger polypeptide sequence such as, for example a full length or mature protein sequence as described by SEQ ID NOS: SEQ ID NOS:36, 37, 38 or 39.

Hereinafter, the term "BCMP-7 or BCMP-11 protein complex" includes protein complexes which comprise a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide as described in (a) or (b), above. In a preferred embodiment, a BCMP-7 protein complex comprises an interacting polypeptide which comprises or consists of the amino acid sequence of SEQ ID NOS: SEQ ID NOS:36, 37 or 38, and a BCMP-11 protein complex comprises an interacting polypeptide which comprises or consists of the amino acid sequence of SEQ ID NOS:36, 37 or 39. The interaction between a BCMP-7 or a BCMP-11 polypeptide and an interacting polypeptide is a direct interaction. The interaction occurs at an interaction domain (or binding domain). An interaction domain preferably comprises at least 4 amino acids and more preferably at least 10 amino acids. The actual interaction domain of a polypeptide is that region or regions of a sequence which contact the interaction domain of another polypeptide. It will be apparent to one skilled in the art that not each and every amino acid within an interaction domain (which is preferably contained within a selected interaction domain; see below) will be involved in an interaction.

Thus, key residues may contribute to an interaction between two proteins, for example but without limitation, interactions may occur between oppositely charged amino acids or via hydrogen bonding or other non-covalent interactions. The function of an interaction domain includes maintaining the interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide, thus facilitating or promoting the formation of a protein complex; *i.e.* the interaction domain is functional. The interaction or binding of a BCMP-7 or BCMP-11 polypeptide with an interacting polypeptide can be assigned an affinity. "Affinity of binding" can be defined as the affinity constant K_a of an interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide of the present invention and has the following mathematical relationship:

$$K_a = \frac{[\text{interacting polypeptide/protein complex}]}{[\text{free interacting polypeptide}]\,[\text{free BCMP-7 or BCMP-11 polypeptide}]};$$

wherein [free interacting polypeptide], [free BCMP-7 or BCMP-11 polypeptide] and [interacting polypeptide /protein complex] consist of the concentrations at equilibrium respectively of the free interacting polypeptide, of the free BCMP-7 or BCMP-11 polypeptide and of the protein complex formed between said interacting polypeptide and said BCMP-7 or BCMP-11 polypeptide.

The affinity of an interaction of the present invention can be assessed, for example but not by way of limitation, using Surface Plasmon Resonance *e.g.* using a Biacore™ apparatus (marketed by Amersham Pharmacia Biotech).

As used herein the phrase "at least the same affinity" with respect to the binding affinity between an interacting polypeptide and a BCMP-7 or BCMP-11 polypeptide means that the K_a is identical or can be at least two-fold, at least three-fold or at least five fold greater than the K_a value of reference.

The BCMP-7 or BCMP-11 protein complex of the invention may be used to find further, as yet unidentified, interacting polypeptides. The BCMP-7 or BCMP-11 protein complexes used to find further interacting polypeptides may interact in a non-covalent manner or the interaction between the BCMP-7 or BCMP-11 polypeptide with an interacting polypeptide may be covalent such that the complex has been, for example and without limitation, cross-linked. Such further interacting polypeptides preferably interact directly with the BCMP-7 or BCMP-11 protein complex, *i.e.* with BCMP-7 or BCMP-11, or the interacting polypeptide comprising the protein complex. In one embodiment, a previously unidentified interacting polypeptide which interacts indirectly with a BCMP-7 or BCMP-11 protein complex is identified. The interaction may be indirect and thus a protein complex may comprise 3 or more polypeptides, at least one of which interacts with a BCMP-7 or BCMP-11 polypeptide directly. A protein complex may alternatively comprise 4 or more polypeptides, *i.e.* a BCMP-7 or BCMP-11 directly interacting with an interacting polypeptide, a third polypeptide which interacts directly with the BCMP-7 or BCMP-11 polypeptide, or directly with the interacting polypeptide of the protein complex, and at least one other

previously unidentified interacting polypeptide which interacts directly or indirectly with said third polypeptide.

In identifying further interactors of a BCMP-7 or BCMP-11 protein complex, the present invention is not limited to the type of method utilized to detect protein-protein interactions. Accordingly, any method known in the art and variants thereof can be used. Preferred methods, however, are described in WO 99/42612 or WO 00/66722, both references incorporated herein by reference, due to the sensitivity of the method and its reproducibility and reliability.

Protein-protein interactions can also be detected using complementation assays such as those described by Pelletier *et al.* at http://www.abrf.org/JBT/Articles/JBT0012/jbt0012.html, WO 00/07038 and WO 98/34120.

Besides the two-hybrid systems, other systems may be used to detect protein-protein interactions. For example, a two-hybrid plus one system has been developed that allows the use of two proteins as bait to screen available cDNA libraries to detect a third partner. This method permits the detection between proteins that are part of a larger protein complex such as the RNA polymerase II holoenzyme and the transcription factors TFIIH or TFIID complexes. Therefore, this method, in general, permits the detection of ternary complex formation as well as inhibitors preventing the interaction between the two previously defined fused proteins (two hybrid plus one or three-hybrid system).

The latter system also allows or prevents the formation of the transcriptional activator since the third partner can be expressed from a conditional promoter such as the methionine-repressed Met25 promoter which is positively regulated in medium lacking methionine. The presence of the methionine-regulated promoter provides an excellent control to evaluate the activation or inhibition properties of the third partner due to its "on" and "off" switch for the formation of the transcriptional activator. An example of the three-hybrid method is described in Tirode *et al.*, 1997, J. Biol. Chem. 272:22995-22999.

Although the above methods are described for applications in the yeast system, the present invention is not limited to detecting protein-protein interactions using yeast, but also includes similar methods that can be used in detecting protein-protein interactions in, for example, mammalian systems as described, for example in Takacs *et al.* (1993, Proc. Natl. Acad. Sci., USA, 90:10375-79) and Vasavada *et al.* (1991, Proc. Natl. Acad. Sci., USA, 88:10686-90), as well as a bacterial two-hybrid system as described in Karimova *et al.* 1998, Proc. Natl. Acad. Sci. USA 95:5752-6, WO 99/28746, WO 00/66722 and Legrain *et al.*, 2000, FEBS Letts. 480:32-36).

The interacting polypeptides in the protein complexes of the invention in their mature form correspond to C4.4A (GenBank Accession Nos. AAD13751; NP_055215; SEQ ID NO:37), alpha-dystroglycan (DAG-1; GenBank Accession No. NP_004384; SEQ ID NO:36), PLAC1 (GenBank Accession Nos. AAG22596; NP_068568; SEQ ID NO:38) and CD68 (Swiss-Prot Accession No. P34810; SEQ ID NO:39). Thus, BCMP-7 and BCMP-11 were found to interact with C4.4A protein (SEQ ID NO: 37), a GPI-anchored metastasis-associated protein, an interaction which has not previously been described. BCMP-7 and BCMP-11 also interacted with DAG-1 (SEQ ID NO:36), a transmembrane protein

associated with cancer. Additionally, BCMP-7, but not BCMP-11, interacted with the PLAC1 antigen (SEQ ID NO: 38), which is predicted to be a secreted protein, and BCMP-11, but not BCMP-7, interacted with CD68 (SEQ ID NO:39), a known transmembrane glycoprotein of the haematopoetic system. The proteins C4.4A, DAG-1, PLAC1 and CD68 are described as "interacting polypeptides".

In the context of the present invention, the following terms are defined: "prey polynucleotide" means a chimeric polynucleotide encoding a polypeptide comprising (i) a specific domain; and (ii) a polypeptide that is to be tested for interaction with a bait polypeptide. The specific domain is, for example, a transcriptional activating domain. A "bait polynucleotide" is a chimeric polynucleotide encoding a chimeric polypeptide comprising (i) a complementary domain; and (ii) a polypeptide that is to be tested for interaction with at least one prey polypeptide. The complementary domain is, for example, a DNA-binding domain that recognizes a binding site that is further detected and is contained in the host organism. A "complementary domain" means a functional constitution of the activity when bait and prey are interacting; for example, enzymatic activity. A "specific domain" means a functional interacting activation domain that can work through different mechanisms by interacting directly or indirectly through intermediary proteins with RNA polymerase II or III-associated proteins in the vicinity of the transcription start site.

The bait polynucleotide, as well as the prey polynucleotide can be prepared according to the methods known in the art such as those described above in the publications and patents. The bait and the prey polynucleotides may be obtained from human cDNA, or variants of cDNA fragment from a human cDNA library, and fragments from the genome or transcriptome of human cells cDNA ranging from about 12 to about 5,000 nucleotides, or about 12 to about 10,000 nucleotides, or from about 12 to about 20,000 nucleotides. The prey polynucleotide is then selected, sequenced and identified.

The polypeptides encoded by the nucleotide inserts of the human cDNA prey library thus prepared are termed "prey polypeptides".

Protein complexes of the invention may be provided in isolated form and include protein complexes that have been purified to at least some extent, such that said complex, or polypeptide comprising said complex, has been removed from the original environment in which it is naturally present.

The term "isolated" is not meant to exclude artificial or synthetic mixtures with other compounds, or the presence of impurities which do not interfere with the interaction or biological activity and which may be present, for example, due to partial purification, addition of stabilizers or mixtures with pharmaceutically acceptable excipients and the like. As such an isolated complex is not necessarily purified. An isolated protein complex, includes a protein complex which is substantially free of those compounds that are normally associated with said complex in a natural state such as other proteins or polypeptides, nucleic acids, carbohydrates, lipids, and the like.

Preferably, the protein complexes of the invention are provided in substantially pure form, that is to say free, to a substantial extent, from other proteins. Purified includes at least

one order of magnitude of purification is achieved, more preferably two or three orders of magnitude are achieved and even more preferably four or five orders of magnitude of purification are achieved. Most preferably, protein complexes are provided in a substantially purified form which is close to 100% pure. Thus, a protein complex may be provided in a composition in which it is the predominant component present (*i.e.* it is present at a level of at least 50%; preferably at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%; when determined on a weight/weight basis excluding solvents or carriers).

In the context of the present invention, the protein complexes of the invention can be obtained from a biological sample from any source, such as and without limitation, a breast or prostate cancer sample. These can be provided by means known in the art including chromatography, immunoprecipitation or other methods known in the art for protein purification which are described in more detail below. For example, an antibody specifically recognising one member of a protein complex may be used to specifically capture a protein complex from a sample, *e.g.* the antibody may be covalently or non-covalently attached to a solid support such as a bead. After capture the protein complex may be eluted from the bead using a suitable buffer. Alternatively, individual members of a protein complex may be captured individually from samples using antibodies which specifically recognise said individual members. The protein complex may then be prepared by mixing the appropriate members, *in vitro*. In another embodiment, protein complexes can be provided in recombinant form. Recombinant complexes can be produced using standard molecular biology techniques known in the art. Examples of such techniques are described below in relation to production of polypeptides and polynucleotides. For example, but without limitation, a protein complex may be provided by the combination of a recombinant sample comprising a BCMP-7 or BCMP-11 polypeptide and a recombinant sample comprising an interacting polypeptide as described herein, by virtue of interaction between the interaction domain or domains present within the sequence of each polypeptide. In another embodiment, a host cell may be co-transfected with vectors comprising polynucleotides encoding a BCMP-7 or BCMP-11 polypeptide and a polynucleotide encoding an interacting polypeptide as described herein. In yet another embodiment, either a BCMP-7 or BCMP-11 polypeptide may be recombinantly expressed within a host cell where said polypeptide forms a protein complex comprising an endogenously present interacting polypeptide, or a recombinantly expressed interacting polypeptide within a host cell forms a protein complex comprising an endogenously present BCMP-7 or BCMP-11.

For examples of protein complex isolation, see Blackstock, W. & Weir, M., 1999, Trends in Biotechnology, 17: 121-127; Rigaut, G. 1999, Nature Biotechnology, 17: 1030-1032; Husi, H. 2000, Nature Neurosci. 3:661-669; Ho, Y. *et al.*, 2002, Nature, 415:180-183; Gavin, A. *et al.*, 2002, Nature, 415: 141-147.

In the context of the present invention, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified and particularly refer to BCMP-7, BCMP-11 and/or interacting polypeptides unless otherwise stated. Polypeptides can include polypeptides with post-translational modifications such as, and without limitation, glycosylation, acetylation, phosphorylation, addition of lipid groups,

and the like. Also encompassed by this definition of "polypeptide" are homologues and orthologues thereof. A homologue includes a polypeptide encoded within a structurally similar gene contained within a given species, while an orthologue includes polypeptides encoded within functionally equivalent genes from a given species or strain, as determined for example, in a standard complementation assay as known in the art.

Amino acid substitutions may be conservative or semi-conservative as known in the art and preferably do not significantly affect the desired activity of the polypeptide. Substitutions may be naturally occurring or may be introduced for example using mutagenesis (*e.g.* Hutchinson *et al.*, 1978, J. Biol. Chem. 253:6551). Thus, the amino acids glycine, alanine, valine, leucine and isoleucine can often be substituted for one another (amino acids having aliphatic side chains). Of these possible substitutions, it is preferred that glycine and alanine are used to substitute for one another (since they have relatively short side chains) and that valine, leucine and isoleucine are used to substitute for one another (since they have larger aliphatic side chains which are hydrophobic). Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains);
- cysteine and methionine (amino acids having sulphur-containing side chains); and
- aspartic acid and glutamic acid can substitute for phospho-serine and phospho-threonine, respectively (amino acids with acidic side chains).

Modifications include naturally occurring modifications such as, without limitation, post-translational modifications and also non-naturally occurring modifications such as may be introduced by mutagenesis.

Thus, BCMP-7, BCMP-11 and interacting polypeptides may be in the form of a 'mature' protein or may be part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, a pre-, pro- or prepro-protein sequence, or a sequence which aids in purification such as an affinity tag, for example, but without limitation, multiple histidine residues, a FLAG tag, HA tag or myc tag. An additional sequence which may provide stability during recombinant production may also be used. Such sequences may be optionally removed as required by incorporating a cleavable sequence as an additional sequence or part thereof. Thus, a BCMP-7, BCMP-11 or interacting polypeptide may be fused to other moieties including other polypeptides. Such additional sequences and affinity tags are well known in the art.

A derivative of a polypeptide, which includes but is not limited to, homologues and orthologues, has one or more amino acid substitutions, modifications, deletions or insertions relative to the amino acid sequences of SEQ ID NOS:1, 2, 13-20 or 35-39, and which retains the interaction domain of BCMP-7, BCMP-11 or interacting polypeptide. Most preferably a derivative of a polypeptide has at least 70% sequence identity to said amino acid sequences, more preferably it has at least 75%, at least 80%, at least 85%, at least

90%, at least 95% or at least 98% identity and which retain a functional interaction domain such that interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide is maintained. Percentage sequence identity refers to the identity between two peptides or between two nucleic acids. Identity between sequences can be determined by comparing a position in each of the sequences which are aligned for the purposes of comparison and is well described in the art. Percentage identity can be calculated using, for example but without limitation, the BLAST™ software available from NCBI (Altschul, S.F. *et al.*, 1990, J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.*, 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.*, 1997, Nucleic Acids Res. 25:3389-3402); Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656).

Recombinant polypeptides may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems. Accordingly, the invention also provides a method of producing a BCMP-7 or BCMP-11 protein complex comprising:

(a) providing an expression system comprising a nucleic acid capable of producing a BCMP-7 polypeptide or a BCMP-11 polypeptide, wherein said expression system is present in a compatible host cell;

(b) providing an expression system comprising a nucleic acid capable of producing an interacting polypeptide, wherein said expression system is present in a compatible host cell;

wherein, under appropriate culture conditions, said host cells produce polypeptides which on combination form the protein complex.

The invention also provides recombinant host cells comprising the expression systems described above. In one embodiment, the expression systems of (a) and (b), above are present within the same host cell.

Accordingly, the present invention also relates to expression systems as described above, to host cells which are genetically engineered with such expression systems and to the production of polypeptides by recombinant techniques. Cell-free translation systems can also be employed to produce recombinant polypeptides (*e.g.* rabbit reticulocyte lysate, wheat germ lysate, SP6/T7 *in vitro* T&T and RTS 100 *E. Coli* HY transcription and translation kits from Roche Diagnostics Ltd., Lewes, UK and the TNT Quick coupled Transcription/Translation System from Promega UK, Southampton, UK.

For recombinant polypeptide production, host cells can be genetically engineered to incorporate expression systems or portions thereof for nucleic acids. Such incorporation can be performed using methods well known in the art, such as, calcium phosphate transfection, DEAD-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection (see *e.g.* Davis *et al.*, Basic Methods in Molecular Biology, 1986 and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbour laboratory Press, Cold Spring Harbour, NY, 1989).

A wide variety of expression systems can be used, such as and without limitation, chromosomal, episomal and virus-derived systems, *e.g.* vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector which is able to maintain, propagate or express a nucleic acid to produce a polypeptide in a host may be used. The appropriate nucleic acid sequence may be inserted into an expression system by any variety of well-known and routine techniques, such as those set forth in Sambrook *et al., supra.* Appropriate secretion signals may be incorporated into the polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Useful expression vectors that can be used include, for example, segments of chromosomal, non-chromosomal and synthetic DNA sequences. Suitable vectors include, but are not limited to, derivatives of SV40 and pcDNA and known bacterial plasmids such as col EI, pCR1, pBR322, pMal-C2, pET, pGEX as described by Smith *et al* (1988), pMB9 and derivatives thereof, plasmids such as RP4, phage DNAs such as the numerous derivatives of phage I such as NM989, as well as other phage DNA such as M13 and filamentous single stranded phage DNA; yeast plasmids such as the 2 micron plasmid or derivatives of the 2m plasmid, as well as centomeric and integrative yeast shuttle vectors; vectors useful in eukaryotic cells such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or the expression control sequences; and the like.

For example in a baculovirus expression system, both non-fusion transfer vectors, such as, but not limited to pVL941 (*Bam*HI cloning site Summers), pVL1393 (*Bam*HI, *Sma*I, *Xba*I, *Eco*RI, *Not*I, *Xma*III, *Bgl*II and *Pst*I cloning sites; Invitrogen), pVL1392 (*Bgl*II, *Pst*I, *Not*I, *Xma*III, *Eco*RI, *Xba*I, *Sma*I and *Bam*HI cloning site; Summers and Invitrogen) and pBlue*Bac*III (*Bam*HI, *Bgl*II, *Pst*I, *Nco*I and *Hind*III cloning site, with blue/white recombinant screening, Invitrogen), and fusion transfer vectors such as, but not limited to, pAc700 (*Bam*HI and *Kpn*I cloning sites, in which the *Bam*HI recognition site begins with the initiation codon; Summers), pAc701 and pAc70-2 (same as pAc700, with different reading frames), pAc360 (*Bam*HI cloning site 36 base pairs downstream of a polyhedrin initiation codon; Invitrogen (1995)) and pBlueBacHisA, B, C (three different reading frames with *Bam*HI, *Bgl*II, *Pst*I, *Nco*I and *Hind*III cloning site, an N-terminal peptide for ProBond purification and blue/white recombinant screening of plaques; Invitrogen (220) can be used.

Mammalian expression vectors contemplated for use in the invention include vectors with inducible promoters, such as the dihydrofolate reductase promoters, any expression

vector with a DHFR expression cassette or a DHFR/methotrexate co-amplification vector such as pED (*Pst*I, *Sal*I, SbaI, SmaI and *Eco*RI cloning sites, with the vector expressing both the cloned gene and DHFR; Kaufman, 1991). Alternatively a glutamine synthetase/methionine sulfoximine co-amplification vector, such as pEE14 (*Hind*III, *Xba*I, *Sma*I, *Sba*I, *Eco*RI and *Bcl*I cloning sites in which the vector expresses glutamine synthetase and the cloned gene; Celltech). A vector that directs episomal expression under the control of the Epstein Barr Virus (EBV) or nuclear antigen (EBNA) can be used such as pREP4 (*Bam*HI, *Sfi*I, *Xho*I, *Not*I, *Nhe*I, *Hind*III, *Nhe*I, *Pvu*I and *Kpn*I cloning sites, constitutive RSV-LTR promoter, hygromycin selectable marker; Invitrogen), pCEP4 (*Bam*HI, *Sfi*I, *Xho*I, *Not*I, *Nhe*I, *Hind*III, *Nhe*I, *Pvu*I and *Kpn*I cloning sites, constitutive hCMV immediate early gene promoter, hygromycin selectable marker; Invitrogen), pMEP4 (*Kpn*I, *Pvu*I, *Nhe*I, *Hind*III, *Not*I, *Xho*I, *Sfi*I, *Bam*HI cloning sites, inducible methallothionein IIa gene promoter, hygromycin selectable marker, Invitrogen), pREP8 (*Bam*HI, *Xho*I, *Not*I, *Hind*III, *Nhe*I and *Kpn*I cloning sites, RSV-LTR promoter, histidinol selectable marker; Invitrogen), pREP9 (*Kpn*I, *Nhe*I, *Hind*III, *Not*I, *Xho*I, *Sfi*I, *Bam*HI cloning sites, RSV-LTR promoter, G418 selectable marker; Invitrogen), and pEBVHis (RSV-LTR promoter, hygromycin selectable marker, N-terminal peptide purifiable via ProBond resin and cleaved by enterokinase; Invitrogen).

Selectable mammalian expression vectors for use in the invention include, but are not limited to, pRc/CMV (*Hind*III, *Bst*XI, *Not*I, *Sba*I and *Apa*I cloning sites, G418 selection, Invitrogen), pRc/RSV (*Hind*III, *Spe*I, *Bst*XI, *Not*I, *Xba*I cloning sites, G418 selection, Invitrogen) and the like. Vaccinia virus mammalian expression vectors (see, for example Kaufman 1991 that can be used in the present invention include, but are not limited to, pSC11 (*Sma*I cloning site, TK- and β-gal selection), pMJ601 (*Sal*I, *Sma*I, *Afl*I, *Nar*I, *Bsp*MII, *Bam*HI, *Apa*I, *Nhe*I, *Sac*II, *Kpn*I and *Hind*III cloning sites; TK- and β-gal selection), pTKgptF1S (*Eco*RI, *Pst*I, *Sal*II, *Acc*I, *Hind*II, *Sba*I, *Bam*HI and *Hpa* cloning sites, TK or XPRT selection) and the like.

Yeast expression systems that can also be used in the present invention include, but are not limited to, the non-fusion pYES2 vector (*Xba*I, *Sph*I, *Sho*I, *Not*I, *Gst*XI, *Eco*RI, *Bst*XI, *Bam*HI, *Sac*I, *Kpn*I and *Hind*III cloning sites, Invitrogen), the fusion pYESHisA, B, C (*Xba*I, *Sph*I, *Sho*I, *Not*I, *Bst*XI, *Eco*RI, *Bam*HI, *Sac*I, *Kpn*I and *Hind*III cloning sites, N-terminal peptide purified with ProBond resin and cleaved with enterokinase; Invitrogen), pRS vectors and the like.

Consequently, mammalian and typically human cells, as well as bacterial, yeast, fungi, insect, nematode and plant cells can be used in the present invention and can be transfected by the nucleic acid or recombinant vector as defined herein.

Examples of suitable cells include, but are not limited to, VERO cells, HELA cells such as ATCC No. CCL2, CHO cell lines such as ATCC No. CCL61, COS cells such as COS-7 cells and ATCC No. CRL 1650 cells, W138, BHK, HepG2, 3T3 such as ATCC No. CRL6361, A549, PC12, K562 cells, HEK 293 cells, Sf9 cells such as ATCC No. CRL1711, Cv1 cells such as ATCC No. CCL70, and Bowes melanoma cells, bacterial cells *e.g. E. Coli* (*e.g.* strain DH5-α), *Streptococci, Staphylococci, Streptomyces, Salmonella typhimurium*

and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells, and plant cells. Further suitable cells that can be used in the present invention include yeast cells such as those of *Saccharomyces,* such as *Saccharomyces cerevisiae.*

If the recombinant polypeptide is secreted into the medium, the medium can be recovered in order to isolate said polypeptide. If produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

Polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including, ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, affinity chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography, molecular sieving chromatography, centrifugation methods, electrophoresis methods and lectin chromatography. In one embodiment, a combination of these methods is used. In another embodiment, high performance liquid chromatography is used. In a further embodiment, an antibody which specifically binds to a polypeptide can be used to deplete a sample comprising a polypeptide of said polypeptide or to purify said polypeptide. Techniques well-known in the art, may be used for refolding to regenerate native or active conformations of the polypeptides when the polypeptides have been denatured during isolation and or purification.

Polynucleotides which encode the polypeptides comprising the protein complexes of the invention can be used to prepare individual DNA vectors which encode individual polypeptides or which encode both a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide. These vectors can be used, without limitation, to express the protein complexes of the invention. Such nucleic acids:

d) comprise or consist of the DNA sequences of SEQ ID NOS:5, 6, 7, 8, 9, 10, 11, 12 or 34) or its RNA equivalent;

e) have a sequence which is complementary to the sequences of d);

f) have a sequence which codes for an interacting polypeptide as described above; or

g) have a sequence which shows substantial identity with any of those of d), e) and f) and which maintains a functional interaction domain.

In particular, the selected interaction domain nucleic acid sequences of SEQ ID NOS:5 to 12 and 34 encode the selected interaction domain amino acid sequences (SEQ ID Nos:13 to 20 and 35) where, for each bait polypeptide, one or more prey polypeptides may be identified. Where more than one prey polypeptide is identified, a selected interaction domain can be identified by comparing and selecting the intersection of every prey polypeptide (see Figure 1). Thus, selected interaction domains are present within an interacting polypeptide. Selected interaction domains do not necessarily interact over their whole length with a BCMP-7 or BCMP-11 polypeptide. It will be apparent to one skilled in the art that selected interaction domain nucleic acid sequences encode polypeptides which have the capacity to interfere with the interaction between a BCMP-7 or BCMP-11

polypeptide and an interacting polypeptide *in vitro or in vivo*. Thus, interaction domain polypeptides (SEQ ID NOS:13 to 20 and 35) encoded by nucleic acid sequences of SEQ ID NOS:5 to 12 and 34) are agents which can modulate the protein complexes of the invention and could potentially be used as a target for therapeutic intervention and thus could be targeted with small molecules or antibodies.

The nucleic acids described in (d) to (g) above can include fragments having at least 12 consecutive nucleic acids, between 12 and 5,000 consecutive nucleic acids and between 12 and 10,000 consecutive nucleic acids and between 12 and 20,000 consecutive nucleic acids, as well as derivatives thereof. The fragments or derivatives of the selected interaction domain sequences comprise at least one interaction domain and preferably possess at least the same affinity of binding to its protein or polypeptide counterpart, against which it has been initially selected. Moreover, this derivative and/or fragment of the selected interaction domain sequences alternatively can have between 95% and 99.999% sequence identity to its polypeptide counterpart.

BCMP-7 and BCMP-11 nucleic acids may be obtained using standard cloning and screening techniques, from a cDNA library derived from mRNA in human cells, using expressed sequence tag (EST) analysis (Adams, M. *et al.*, 1991, Science, 252:1651-1656; Adams, M. *et al.*, 1992, Nature 355:632-634; Adams, M. *et al.*, 1995, Nature, 377:Suppl: 3-174). Nucleic acids can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques. The nucleic acids comprising coding sequence for BCMP-7, BCMP-11 or interacting polypeptides can be used for the recombinant production of said polypeptides. Such nucleic acids may include the coding sequence for the mature polypeptide, by itself; or the coding sequence for the mature polypeptide in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, pro- or prepro-protein sequence, a cleavable sequence or other fusion peptide portions, such as an affinity tag or an additional sequence conferring stability during production of the polypeptide. Preferred affinity tags include multiple histidine residues (for example see Gentz *et al.*, 1989, Proc. Natl. Acad. Sci USA 86:821-824), a FLAG tag, HA tag or myc tag. Such nucleic acids may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Polypeptide derivatives as referred to above can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of a nucleic acid of the invention such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Standard techniques known to those of skill in the art can be used to introduce mutations, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues.

A nucleic acid encoding a BCMP-7 or BCMP-11, including homologues and orthologues from species other than human, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridisation conditions with a labelled probe having the sequence of such a nucleic acid as described in

d)-g) above, and isolating full-length cDNA and genomic clones containing said nucleic acid sequence. Such hybridisation techniques are well-known in the art. One example of stringent hybridisation conditions is where attempted hybridisation is carried out at a temperature of from about 35°C to about 65°C using a salt solution of about 0.9M. However, the skilled person will be able to vary such conditions as appropriate in order to take into account variables such as probe length, base composition, type of ions present, etc. For a high degree of selectivity, relatively stringent conditions such as low salt or high temperature conditions, are used to form the duplexes. Highly stringent conditions include hybridisation to filter-bound DNA in 0.5M $NaHPO_4$, 7% sodium dodecyl sulphate (SDS), 1mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. *et al.*, eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, at p. 2.10.3). For some applications, less stringent conditions for duplex formation are required. Moderately stringent conditions include washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel *et al.*, 1989, *supra*). Hybridisation conditions can also be rendered more stringent by the addition of increasing amounts of formamide, to destabilise the hybrid duplex. Thus, particular hybridisation conditions can be readily manipulated, and will generally be chosen as appropriate. In general, convenient hybridisation temperatures in the presence of 50% formamide are: 42°C for a probe which is 95-100% identical to the fragment of a gene encoding a polypeptide as defined herein, 37°C for 90-95% identity and 32°C for 70-90% identity.

One skilled in the art will understand that, in many cases, an isolated cDNA sequence will be incomplete, in that the region coding for the polypeptide is cut short at the 5' end of the cDNA. This is a consequence of reverse transcriptase, an enzyme with inherently low processivity (a measure of the ability of the enzyme to remain attached to the template during the polymerization reaction), failing to complete a DNA copy of the mRNA template during first strand cDNA synthesis.

Methods to obtain full length cDNAs or to extend short cDNAs are well known in the art, for example RACE (Rapid amplification of cDNA ends; *e.g.* Frohman *et al.*, 1988, Proc. Natl. Acad. Sci USA 85:8998-9002). Recent modifications of the technique, exemplified by the Marathon™ technology (Clontech Laboratories Inc.) have significantly simplified the search for longer cDNAs. This technology uses cDNAs prepared from mRNA extracted from a chosen tissue followed by the ligation of an adaptor sequence onto each end. PCR is then carried out to amplify the missing 5'end of the cDNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using nested primers which have been designed to anneal with the amplified product, typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the known gene sequence. The products of this reaction can then be analysed by DNA sequencing and a full length cDNA constructed either by joining the product directly to the existing cDNA to give a complete sequence, or carrying out a separate full length PCR using the new sequence information for the design of the 5' primer.

Nucleic acids encoding BCMP-7 and BCMP-11 polypeptides and interacting polypeptides of the invention may be provided using any means known in the art as discussed further below.

Such nucleic acids can include those nucleic acid molecules defined in d) to g) above as well as nucleic acids encoding BCMP-7 and BCMP-11 polypeptides and interacting polypeptidesand may have one or more of the following characteristics:

1) they may be DNA or RNA;
2) they may be single or double stranded;
3) they may be in substantially pure form. Thus, they may be in a form which is substantially free from contaminating proteins and/or from other nucleic acids; and
4) they may be with introns or without introns (*e.g.* as cDNA).

Nucleic acids encoding a selected interaction domain could also be used in the preparation of a pharmaceutical composition for use in the treatment of cancer and could be administered via gene therapy (see for example Hoshida, T. *et al.,* 2002, Pancreas, 25:111-121; Ikuno, Y. 2002, Invest. Ophthalmol. Vis. Sci. 2002 43:2406-2411; Bollard, C., 2002, Blood 99:3179-3187; Lee E., 2001, Mol. Med. 7:773-782; Asoh, S. *et al.*, 2002, Proc. Natl. Acad. Sci. USA, 99:17107-17112; Goldspiel *et al.,* 1993, Clin. Pharm. 12:488-505).

A further aspect of the invention relates to a vaccine composition of use in the treatment of cancer. A BCMP-7 or BCMP-11 protein complex can be used in the production of vaccines for treatment of cancer. Such material can be antigenic and/or immunogenic. Antigenic includes a protein or nucleic acid that is capable of being used to raise antibodies or indeed is capable of inducing an antibody response in a subject. Immunogenic material includes a protein or nucleic acid that is capable of eliciting an immune response in a subject. Thus, in the latter case, the protein or nucleic acid may be capable of not only generating an antibody response but, in addition, a non-antibody based immune responses *i.e.* a cellular or humoral response.

Since a polypeptide or a nucleic acid may be broken down in the stomach, the vaccine composition is preferably administered parenterally (*e.g.* subcutaneous, intramuscular, intravenous or intradermal injection).

Accordingly, in further embodiments, the present invention provides:

a) the use of such a vaccine in inducing an immune response in a subject; and
b) a method for the treatment of cancer in a subject, or of vaccinating a subject against cancer which comprises the step of administering to the subject an effective amount of a protein complex, preferably as a vaccine.

BCMP-7 and BCMP-11 protein complexes also find use in raising antibodies. Thus, the present invention provides an antibody that specifically recognises a BCMP-7 or BCMP-11 protein complex, wherein the antibody does not recognise a BCMP-7 polypeptide or a BCMP-11 polypeptide or an interacting polypeptide when said BCMP-7 or BCMP-11 or interacting polypeptide is in an uncomplexed form. The antibodies of the invention do not

specifically bind to a BCMP-7, BCMP-11 or an interacting polypeptide, but only specifically bind to, or recognise, a protein complex comprising a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide.

Thus, protein complexes of the invention can be used as an immunogen to generate antibodies which specifically bind such an immunogen. Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments and F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.* molecules that contain an antigen binding site that specifically binds an antigen. The immunoglobulin molecules of the invention can be of any class (*e.g.* IgG, IgE, IgM, IgD and IgA) or subclass of immunoglobulin molecule.

In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.* ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a protein complex of the invention, one may assay generated hybridomas for a product which binds to said protein complex but which does not bind to individual polypeptides which comprise the complex when said polypeptides are present in isolation (*i.e.* in the absence of other members of the protein complex). For selection of an antibody that specifically binds a first protein complex but which does not specifically bind to (or binds less avidly to) a second protein complex, one can select on the basis of positive binding to the first complex and a lack of binding to (or reduced binding to) the second complex.

Antibodies which recognise a BCMP-7 polypeptide, a BCMP-11 polypeptide or an interacting polypeptide are of use in the preparation of a protein complex. Thus, a sample comprising a polypeptide can be specifically depleted of said polypeptide by an antibody recognising said polypeptide. The polypeptide can then be eluted from said antibody using means known in the art, such as using 0.1M glycine at low pH.

For preparation of monoclonal antibodies (mAbs), any technique which provides for the production of antibody molecules by continuous cell lines in culture can be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). The hybridoma producing the mAbs of the invention can be cultivated *in vitro* or *in vivo*. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing known technology.

The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (*e.g.* human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region

derived from a murine mAb (see, *e.g.* US 4,816,567 and US 4,816397). Humanized antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule (see, *e.g.* US 5,585,089).

Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in WO 87/02671; EP 184,187; EP 171,496; EP 173,494; WO 86/01533; US 4,816,567; EP 125,023; Better *et al.*, 1988, Science 240:1041-1043; Liu *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu *et al.*, 1987, J. Immunol. 139:3521-3526; Sun *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura *et al.*, 1987, Canc. Res. 47:999-1005; Wood *et al.*, 1985, Nature 314:446-449; and Shaw *et al.*, 1988, J. Natl. Cancer Inst. 80:1553-1559; Morrison, 1985, Science 229:1202-1207; Oi *et al.*, 1986, Bio/Techniques 4:214; US 5,225,539; Jones *et al.*, 1986, Nature 321:552-525; Verhoeyan *et al.* (1988) Science 239:1534; and Beidler *et al.*, 1988, J. Immunol. 141:4053-4060.

Completely human antibodies are useful for therapeutic treatment of human patients. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.* protein complex of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harboured by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg & Huszar (1995, Int. Rev. Immunol. 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, *e.g.* US 5,625,126; US 5,633,425; US 5,569,825; US 5,661,016; and US 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, *e.g.* a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (Jespers *et al.*, 1994, Bio/technology 12:899-903).

The antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. More specifically, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (*e.g.* human or murine). Phage expressing an antigen binding domain that binds the antigen of interest

can be selected or identified with antigen, *e.g.* using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman *et al.*, J. Immunol. Methods 182:41-50 (1995); Ames *et al.*, J. Immunol. Methods 184:177-186 (1995); Kettleborough *et al.*, Eur. J. Immunol. 24:952-958 (1994); Persic *et al.*, Gene 187 9-18 (1997); Burton *et al.*, Advances in Immunology 57:191-280 (1994); EP0585287; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.* as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax *et al.*, BioTechniques 12(6):864-869 (1992); and Sawai *et al.*, AJRI 34:26-34 (1995); and Better *et al.*, Science 240:1041-1043 (1988).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in US 4,946,778 and 5,258,498; Huston *et al.*, Methods in Enzymology 203:46-88 (1991); Shu *et al.*, PNAS 90:7995-7999 (1993); and Skerra *et al.*, Science 240:1038-1040 (1988).

The invention further provides bispecific antibodies, which can be made by methods known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein *et al.*, 1983, Nature 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 and in Traunecker *et al.*, 1991, EMBO J. 10:3655-3659.

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. In an embodiment, the fusion is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. Generally, the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This

provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In another embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details for generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology, 1986, 121:210.

The invention provides functionally active fragments, derivatives or analogs of the anti-polypeptide immunoglobulin molecules. Functionally active means that the fragment, the derivative or the analog is able to elicit anti-anti-idiotype antibodies (*i.e.* tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, the derivative or the analog is derived. Specifically, in an embodiment, the antigenicity of the idiotype of the immunoglobulin molecule is enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

The present invention provides antibody fragments such as, but not limited to, F(ab')2 fragments and Fab fragments. Antibody fragments which recognize specific epitopes can be generated by known techniques. F(ab')2 fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the F(ab')2 fragments. The invention also provides heavy chain and light chain dimmers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (*e.g.* as described in US 4,946,778; Bird, 1988, Science 242:423-42; Huston *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward *et al.*, 1989, Nature 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* can be used (Skerra *et al.*, 1988, Science 242:1038-1041).

In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (*e.g.* a peptide bond), at either the N-terminus

or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. In an embodiment, the immunoglobulin, or a fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins can facilitate purification, increase half-life *in vivo*, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

The immunoglobulins of the invention include analogues and derivatives that are either modified, *i.e.* by the covalent attachment of any type of molecule as long as such covalent attachment that does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogues of the immunoglobulins include those that have been further modified, *e.g.* by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analogue or derivative can contain one or more non-classical amino acids.

The foregoing antibodies can be used in methods known in the art relating to the localization and activity of BCMP-7 and BCMP-11 protein complexes, *e.g.* for imaging or radioimaging these complexes, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc. and for radiotherapy.

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, for instance, by chemical synthesis or by recombinant expression. In an embodiment, they are produced by recombinant expression technique.

Recombinant expression of antibodies, or fragments, derivatives or analogues thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody can be assembled from chemically synthesized oligonucleotides (*e.g.* as described in Kutmeier *et al.*, 1994, BioTechniques 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, the nucleic acid encoding the antibody can be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody can be obtained from a suitable source (*e.g.* an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate

polyclonal antibodies or, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody can be obtained by screening Fab expression libraries (*e.g.* as described in Huse *et al.*, 1989, Science 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (see, *e.g.* Clackson *et al.*, 1991, Nature 352:624; Hane *et al.*, 1997 Proc. Natl. Acad. Sci. USA 94:4937).

In addition, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, 1984, Proc. Natl. Acad. Sci. 81:851-855; Neuberger *et al.*, 1984, Nature 312:604-608; Takeda *et al.*, 1985, Nature 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used.

In another embodiment, antibodies of the invention or fragments thereof are conjugated to a diagnostic or therapeutic moiety. The antibodies can be used for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and non-radioactive paramagnetic metal ions. See generally US 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I, ^{131}I, ^{111}In and ^{99}Tc.

Antibodies of the invention or fragments thereof can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety can be a protein or polypeptide possessing a desired biological activity. Such proteins include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumour necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, *e.g.* Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in Controlled Drug

Delivery (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, 1982, Immunol. Rev. 62:119-58.

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described in US 4,676,980.

An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

In yet another aspect, the present invention provides an agent which modulates the interaction of a BCMP-7 polypeptide, or a BCMP-11 polypeptide, and an interacting polypeptide. Agents identified through the screening methods of the invention are potential therapeutics for use in the treatment of cancer. The agent may be identified by methods known in the art.

The term "agent which modulates an interaction" means an agent which controls or regulates, or can act on another protein which can control or regulate, the protein-protein interaction of a protein complex of at least two polypeptides or the protein-protein interaction of at least two polypeptides. The term "agent which modulates an interaction" also includes an agent which inhibits or promotes, or can act on another protein which can inhibit or promote, the protein-protein interaction of a complex of at least two polypeptides or the protein-protein interaction of at least two polypeptides.

An agent which modulates the interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide does not, for example, have to be 100% effective in modulating the relevant interaction. Partial modulation (*e.g.* disruption, activation or inhibition) can be therapeutically relevant. These modulating agents can act as cofactors, as inhibitors, as antibodies, as tags, as competitive inhibitors, as activators, disruptors or alternatively have agonistic or antagonistic effects on the protein-protein interactions.

Thus, in one aspect, the present invention relates to a method of selecting agents which modulate the interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide of the invention.

In the context of the present invention 'contacting' includes contact with a polypeptide which may be present in a sample, *i.e. in vitro*, or present within a subject such as a diseased, treated or control subject, *i.e. in vivo*. Thus, in the latter case the subject is administered with an antibody of the invention.

Accordingly, provided is a method of modulating the activity of a BCMP-7 polypeptide or a BCMP-11 polypeptide, comprising contacting said polypeptide with an interacting polypeptide of the invention, or an antibody of the invention.

Thus, also provided is a method of modulating the activity of an interacting polypeptide of the invention, comprising contacting said polypeptide with a BCMP-7 or a BCMP-11 polypeptide, or an antibody of the invention.

Further provided is a method of screening for/or identifying an agent that modulates the interaction of a BCMP-7, or a BCMP-11 polypeptide, with an interacting polypeptide, comprising:

(a) contacting a protein complex comprising the BCMP-7 polypeptide or the BCMP-11 polypeptide and the interacting polypeptide of the invention with a candidate agent; and

(b) determining whether the agent modulates the interaction.

In another embodiment, provided is a method of screening for/or identifying an agent that modulates the interaction of a BCMP-7 polypeptide or a BCMP-11 polypeptide with an interacting polypeptide, comprising:

(a) contacting a BCMP-7 or BCMP-11 protein complex with a candidate agent;

(b) contacting a BCMP-7 or BCMP-11protein complex with a control agent; and

(c) determining whether the candidate agent modulates the interaction by comparing the amount of complex present in part (a) with that present in part (b).

In one embodiment, the amount of protein complex present is determined using an antibody which specifically recognises said protein complex comprising a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide.

In another embodiment, the systems described by Vidal *et al.* (1996, Proc. Nat. Acad. Sci. 93:10315-10320) called the reverse two- and one-hybrid systems, can be used to detect protein-protein interactions. Here a collection of molecules can be screened that inhibit a specific protein-protein or protein-DNA interactions, respectively.

In yet another embodiment, an agent which modulates the interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide can be selected according to a method which comprises:

(a) cultivating a recombinant host cell with a candidate agent on a selective medium and a reporter gene the expression of which is toxic for said recombinant host cell wherein said recombinant host cell is transformed with two vectors:

(i) wherein said first vector comprises a bait polynucleotide encoding a first hybrid polypeptide having a DNA-binding domain;

(ii) wherein said second vector comprises a prey polynucleotide encoding a second hybrid polypeptide having a transcriptional activating domain that activates said toxic reporter gene when the first and second hybrid polypeptides interact;

(b) selecting said candidate agent which inhibits or permits the growth of said recombinant host cell.

Thus, the present invention provides agents which modulate the interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide, that control or regulate, or can act on another modulating compound which can in turn control or regulate the protein-protein interactions of a complex comprising a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide.

In another embodiment, the present invention also provides agents which modulate the interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide, that inhibit or promote, or can act on another modulating agent which can in turn inhibit or promote the protein-protein interactions of a protein complex comprising a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide

In a further embodiment, the present invention provides a method of selecting an agent which modulates the interaction between a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide, which inhibits or promotes, or can act on another modulating compound which can in turn inhibit or promote the protein-protein interactions of a protein complex comprising a BCMP-7 or BCMP-11 polypeptide and an interacting polypeptide comprising:

(a) cultivating a recombinant host cell with a candidate agent on a selective medium and a reporter gene the expression of which is toxic for said recombinant host cell wherein said recombinant host cell is transformed with two vectors:

(i) wherein said first vector comprises a bait polynucleotide encoding a first hybrid polypeptide having a first domain of an enzyme;

(ii) wherein said second vector comprises a prey polynucleotide encoding a second hybrid polypeptide having an enzymatic transcriptional activating domain that activates said toxic reporter gene when the first and second hybrid polypeptides interact;

(b) selecting said candidate agent which inhibits or permits the growth of said recombinant host cell.

In the two methods described above any toxic reporter gene can be utilized including those reporter genes that can be used for negative selection including the URA3 gene, the CYH1 gene, the CYH2 gene and the like, and the activating domain can be p42 Gal 4, YP16 (HSV) and the DNA-binding domain can be derived from Gal4 or Lex A. The protein or enzyme can be adenylate cyclase, guanylate cyclase, DHFR, and the like.

In yet another embodiment, the present invention provides a kit for screening for a candidate agent. This kit comprises a recombinant host cell which comprises a reporter gene the expression of which is toxic for the recombinant host cell. The host cell is transformed with two vectors. The first vector comprises a polynucleotide encoding a first hybrid polypeptide having a DNA binding domain; and the second vector comprises a polynucleotide encoding a second hybrid polypeptide having a transcriptional activating domain that activates said toxic reporter gene when the first and second hybrid polypeptides interact.

In yet another embodiment, a kit is provided for screening a modulating compound by providing a recombinant host cell, as described in above, but instead of a DNA binding

domain, the first vector encodes a first hybrid polypeptide containing a first domain of a protein. The second vector encodes a second polypeptide containing a second part of a complementary domain of a protein that activates the toxic reporter gene when the first and second hybrid polypeptides interact.

In yet another embodiment, the present invention relates to protein chips or protein arrays. Microarrays are well known in the art (see Walter *et al.* Curr. Opin. Microbiol. 2000, 3:298-302; MacBeath and Schreiber, 2000, Science, 289:1760-1763; Service, 2000, Science 289: 1673). Ideal surfaces for attachment of proteins include, but are not limited to, filter membranes, polystyrene film, and glass slides coated with, for example, poly-lysine, an aldehyde-containing reagent that attaches to primary amines, or polyacrylamide. Microarray production is highly automated using commercially available pin-based or microdispensing liquid handling robots. Ligand binding is generally detected using fluorescence labelling (but is not limited to this) and the analysis of results performed using commercially available software packages.

Accordingly, provided is a protein array comprising a suitable substrate displaying a plurality of discrete regions each comprising a BCMP-7 and/or BCMP-11 protein complex. These arrays can be prepared using methods known in the art. Such a protein microarray can be used to screen for compounds which modulate the interaction between a BCMP-7 or BCMP-11 polypeptide and its interacting protein. In one embodiment, one or more BCMP-7 and/or BCMP-11 polypeptides are immobilised to individual discrete regions on a substrate using methods known in the art; addition of sample comprising interacting polypeptides produces an array of BCMP-7 and/or BCMP-11 protein complexes. In another embodiment, one or more interacting polypeptides are immobilised to individual discrete regions on a substrate using methods known in the art; addition of sample comprising BCMP-7 and/or BCMP-11 produces an array of BCMP-7 and/or BCMP-11 protein complexes polypeptides. If desired the BCMP-7 or BCMP-11 polypeptides and interacting polypeptides may be covalently linked or cross-linked using means known in the art, *e.g.* using glutaraldehyde. Such covalently linked complexes may, without limitation, be useful for screening for antibodies which bind to a protein complex. In another embodiment, protein complexes may be preformed, optionally covalently cross-linked and be immobilised on a substrate using means known in the art.

In another embodiment, agents that modulate the activity, level or expression of a protein complex are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represents a model of cancer. Accordingly, a first and second group of mammals are administered with a candidate agent or a control agent and the ability of the candidate agent to modulate the formation, activity or level of the protein complex is determined by comparing the difference in the formation, activity or level of expression between the first and second group of mammals. Where desired, the formation, activity or expression levels of the protein complex in the first and second groups of mammals can be compared to that in a control group of mammals. The candidate agent or a control agent can be administered by means known in the art (*e.g.* orally, rectally or parenterally such as

intraperitoneally or intravenously). Changes in the formation, activity or expression of a protein complex can be assessed by the methods outlined herein. In a particular embodiment, a therapeutically effective amount of an agent can be determined by monitoring an amelioration or improvement in disease symptoms, to delay onset or slow progression of the disease, for example but without limitation, a reduction in tumour size. Techniques known to physicians familiar with breast cancer can be used to determine whether a candidate agent has altered one or more symptoms associated with the disease.

One skilled in the art will also appreciate that a BCMP-7 or BCMP-11 protein complex may also be used in a method for the structure-based design of an agent, in particular a small molecule which acts to modulate (*e.g.* stimulate or inhibit) the activity of said complex, said method comprising:

1) determining the three-dimensional structure of said protein complex;
2) deducing the three-dimensional structure within the protein complex of the likely reactive or binding site(s) of the agent;
3) synthesising candidate agents that are predicted to react or bind to the deduced reactive or binding site; and
4) testing whether the candidate agent is able to modulate the binding or activity of said protein complex.

It will be appreciated that the method described above is likely to be an iterative process.

This invention thus further provides agents which modulate the interaction of a BCMP-7 or BCMP-11 protein complex, interacting polypeptides and antibodies, and uses thereof for treatments as described herein. Hereinafter, the latter are referred to as 'active agents'. The term 'treatment' includes either therapeutic or prophylactic therapy. When a reference is made herein to a method of treating or preventing a disease or condition using a particular active agent or combination of agents, it is to be understood that such a reference is intended to include the use of that active agent or combination of agents in the preparation of a medicament for the treatment or prevention of the disease or condition.

Accordingly, the present invention provides a method for the prophylaxis and/or treatment of cancer, which comprises administering to said subject a therapeutically effective amount of at least one active agent of the invention.

In order to use active agents of the invention in therapy (human or veterinary), they will normally be formulated into a pharmaceutical composition in accordance with standard pharmaceutical practice, *e.g.* by admixing the active agent and a pharmaceutically acceptable carrier. Thus, according to a further aspect of the invention there is provided a pharmaceutical composition comprising at least one active agent of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions are particularly useful in the prevention or treatment of cancer, *e.g.* breast or prostate cancer. In one aspect, the pharmaceutical composition is for use as a vaccine and so any additional components will be acceptable for vaccine use. In addition, the skilled person will appreciate that one or more suitable adjuvants may be added to such vaccine preparations.

A pharmaceutically acceptable amount of an active agent can be estimated from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range of an active agent that includes or encompasses a concentration point or range having the desired effect in an *in vitro* system. Thus, this information can be used to accurately determine the doses in mammals, including humans and animals.

A therapeutically effective dose refers to that amount of the active agent that results in amelioration of symptoms in a patient. Toxicity and therapeutic efficacy of such active agents can be determined by standard pharmaceutical procedures in cell cultures or in experimental animals. For example, the LD50 (the dose lethal to 50% of the population) as well as the ED50 (the dose therapeutically effective in 50% of the population) can be determined using methods known in the art. The dose ratio between toxic and therapeutic effects is the therapeutic index which can be expressed as the ratio between LD50 and ED50 compounds that exhibit high therapeutic indexes.

The data obtained from the cell culture and animal studies can be used in formulating a range of dosage of such active agents which lies for instance within a range of circulating concentrations that include the ED50 with little or no toxicity.

The dosage to be administered of an active agent will vary according to the particular active agent, the cancer involved, the subject, and the nature and severity of the disease and the physical condition of the subject, and the selected route of administration; the appropriate dosage can be readily determined by a person skilled in the art. For the treatment of cancer in humans and animals, the dosage may range from 0.01 mg/kg to 750 mg/kg. For prophylactic use in human and animals, the dosage may range from 0.01 mg/kg to 100 mg/kg.

The active agents may be administered in combination, *e.g.* simultaneously, sequentially or separately, with one or more other therapeutically active, *e.g.* anti-tumour, compounds.

The compositions may contain from 0.1% by weight, preferably from 10-60% by weight, of the active agent of the invention, depending on the method of administration.

Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose. Such a unit may contain for example but without limitation, 100mg/kg to 0.1mg/kg depending on the condition being treated, the route of administration and the age, weight and condition of the subject. Preferred unit dosage compositions are those containing a daily dose or sub-dose, as recited above, or an appropriate fraction thereof, of the active ingredient.

It will be recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of an active agent of the invention will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular subject being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of skill in the art that the optimal course of treatment, *i.e.* the number of doses of an active agent of the invention given per

day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Dosage regimens are adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

Pharmaceutically acceptable carriers for use in the invention may take a wide variety of forms depending, *e.g.* on the route of administration.

Compositions for oral administration may be liquid or solid. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Oral liquid preparations may contain suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; water; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid; flavoring agents, preservatives, coloring agents and the like may be used.

In the case of oral solid preparations such as powders, capsules and tablets, carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like may be included. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are generally employed. In addition to the common dosage forms set out above, active agents of the invention may also be administered by controlled release means and/or delivery devices. Tablets and capsules may comprise conventional carriers or excipients such as binding agents for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated by standard aqueous or non-aqueous techniques according to methods well known in normal pharmaceutical practice.

Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient, as a powder or granules, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association the active ingredient with the carrier, which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the

product into the desired presentation. For example, a tablet may be prepared by compression or moulding, optionally with one or more accessory ingredients.

Compressed tablets may be prepared by compressing, in a suitable machine, the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Moulded tablets may be made by moulding, in a suitable machine, a mixture of the powdered compound moistened with an inert liquid diluent. Desirably, each tablet contains from about 1mg to about 500mg of the active ingredient and each cachet or capsule contains from about 1 to about 500mg of the active ingredient.

Compositions comprising an active agent of the invention may also be prepared in powder or liquid concentrate form. Conventional water soluble excipients, such as lactose or sucrose, may be incorporated in the powders to improve their physical properties. Thus, particularly suitable powders of this invention comprise 50 to 100% w/w, and preferably 60 to 80% w/w of the combination and 0 to 50% w/w and preferably 20 to 40% w/w of conventional excipients. When used in a veterinary setting such powders may be added to animal feedstuffs, for example by way of an intermediate premix, or diluted in animal drinking water.

Liquid concentrates of this invention for oral administration suitably contain a water-soluble compound combination and may optionally include a veterinarily acceptable water miscible solvent, for example polyethylene glycol, propylene glycol, glycerol, glycerol formal or such a solvent mixed with up to 30% v/v of ethanol. The liquid concentrates may be administered to the drinking water of animals, particularly poultry.

Pharmaceutical compositions suitable for parenteral administration may be prepared as solutions or suspensions of the active agents of the invention in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include aqueous or non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. Extemporaneous injection solutions, dispersions and suspensions may be prepared from sterile powders, granules and tablets.

The compositions may be presented in unit-dose or multi-dose containers, for example in sealed ampoules and vials and to enhance stability, may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. The sterile liquid carrier may be supplied in a separate vial or ampoule and can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.* glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils. Advantageously, agents such as a

local anaesthetic, preservative and buffering agents can be included the sterile liquid carrier.

In certain embodiments, the active agents of the invention can be formulated to ensure proper distribution in vivo, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.* US 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.* Ranade, V. 1989, J. Clin. Pharmacol. 29: 685).

Exemplary targeting moieties include folate or biotin (see, *e.g.*, US 5,416,016); mannosides (Umezawa *et al.*, 1988, Biochem. Biophys. Res. Comm. 153:1038); antibodies (Bloeman, P. *et al.*, 1995, FEBS Lett. 357:140; Owais, M. *et al.* (1995) Antimicrob. Agents Chemother. 39: 180); surfactant protein A receptor (Briscoe *et al.* (1995) Am. J. Physiol. 1233: 134), different species of which may comprise the compositions of the inventions, as well as components of the invented molecules; psi 20 (Schreier *et al.* (1994) J. Biol. Chem. 269: 9090); see also Keinanen, K. & Laukkanen, M., 1994, FEBS Lett. 346: 123; Killion, J. & Fidler, I., 1994, Immunomethods 4: 273. In one embodiment of the invention, the active agents of the invention are formulated in liposomes; in a more preferred embodiment, the liposomes include a targeting moiety. In a most preferred embodiment, the therapeutic compounds in the liposomes are delivered by bolus injection to a site proximal to the tumour.

Pharmaceutical compositions adapted for topical administration may be formulated as ointments, creams, suspensions, lotions, powders, solutions, pastes, gels, impregnated dressings, sprays, aerosols or oils, transdermal devices, dusting powders, and the like. These compositions may be prepared via conventional methods containing the active ingredient. Thus, they may also comprise compatible conventional carriers and additives, such as preservatives, solvents to assist drug penetration, emollients in creams or ointments and ethanol or oleyl alcohol for lotions. Such carriers may be present as from about 1% up to about 98% of the composition. More usually they will form up to about 80% of the composition. As an illustration only, a cream or ointment is prepared by mixing sufficient quantities of hydrophilic material and water, containing from about 5-10% by weight of the compound, in sufficient quantities to produce a cream or ointment having the desired consistency.

Pharmaceutical compositions adapted for transdermal administration may be presented as discrete patches intended to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. For example, the active ingredient may be delivered from the patch by iontophoresis.

For applications to external tissues, for example the mouth and skin, the compositions are preferably applied as a topical ointment or cream. When formulated in an ointment, the active ingredient may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredient may be formulated in a cream with an oil-in-water cream base or a water-in-oil base.

Pharmaceutical compositions adapted for topical administration in the mouth include lozenges, pastilles and mouth washes.

Pharmaceutical compositions adapted for topical administration to the eye include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent. They also include topical ointments or creams as above.

Pharmaceutical compositions suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter or other glyceride or materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the combination with the softened or melted carrier(s) followed by chilling and shaping moulds. They may also be administered as enemas.

Pharmaceutical compositions adapted for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray compositions. These may comprise emollients or bases as commonly used in the art.

Another aspect of the invention relates to the detection of a BCMP-7 or BCMP-11 protein complex in the diagnosis of cancer in a subject. Accordingly, provided is a diagnostic kit, comprising a capture reagent (*e.g.* an antibody) against a BCMP-7 or BCMP-11 protein complex, instructions for using the capture reagent for diagnosis, prognosis, therapeutic monitoring or any combination of these applications. In addition, such a kit may also comprise one or more of the following:

- a labelled binding partner to the capture reagent;
- a solid phase (such as a reagent strip) upon which the capture reagent is immobilised; and
- a label or insert indicating regulatory approval for diagnostic, prognostic or therapeutic use or any combination thereof.

A capture reagent includes a compound capable of binding to a BCMP-7 or BCMP-11 complex. If no labelled binding partner to the capture reagent is provided, the capture reagent itself can be labelled with a detectable marker, *e.g.* a chemiluminescent, enzymatic, fluorescent, or radioactive moiety (see above).

The methods of diagnosis according to the present invention may be performed using a number of methods known to those skilled in the art, including, without limitation, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, 2 dimensional gel electrophoresis, competitive and non-competitive assay systems using immunoassays described above.

Thus, in another aspect, the invention relates to methods for screening and/or diagnosis of cancer in a subject, and/or monitoring the effectiveness of a cancer therapy, comprising measuring the level or activity of a BCMP-7 or BCMP-11 protein complex in a biological sample obtained from said subject.

In a particular embodiment, the presence of a protein complex within a sample is compared with its presence within another sample, for example the presence of a protein complex may be compared within a sample from a diseased subject with a sample from a

subject free of disease or a treated subject. In another embodiment, the presence of the protein complex is compared to a reference range or control presence. The presence of a protein complex may be described as a level or quantity, or may be described as an activity.

In a further aspect, the method of detecting the presence of a BCMP-7 or BCMP-11 protein complex, comprises detecting the protein complex using a directly or indirectly labelled detection reagent, for example but without limitation, an antibody which recognises the protein complex.

The presence of a protein complex can be detected by means of any immunoassay known in the art, including, without limitation, Western blots, immunocytochemistry, immunohistochemistry, immunoassays, *e.g.* radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

In the context of the present invention, the term cancer includes a malignant new growth that arises from epithelium, found in skin or, more commonly, the lining of body organs, for example: breast, prostate, lung, colon, kidney, pancreas, stomach or bowel. Cancers tend to infiltrate into adjacent tissue and spread (metastasise) to distant organs, for example: to bone, liver, lung or the brain. In the methods of the invention the cancer is preferably prostate cancer and more preferably breast cancer.

Preferred features of each embodiment of the invention are as for each of the other embodiments *mutatis mutandis*. All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The invention will now be described with reference to the following examples, which are merely illustrative and should not in any way be construed as limiting the scope of the present invention. The examples refer to the figure in which:

Fig. 1 is a schematic representation identifying the selected interacting domains of BCMP-7 or BCMP-11 interacting polypeptides. In this figure the "full-length prey protein" is the Open Reading Frame (ORF) or coding sequence (CDS) where the identified prey polypeptides are included. The selected interaction domain is determined by the commonly shared polypeptide domain of every selected prey fragment.

EXAMPLES

Example 1: Endosomal localisation of BCMP-11 in breast cancer cell lines

C-terminal tagging with SuperGlo™ green fluorescent protein (GFP) and immunocytochemistry were used to determine the cellular localisation of BCMP-11 in MDA-MB-468 and T-47D cell lines.

The BCMP-11 full length ORF was PCR cloned into the pQBI25/50-fN1 vector (Qbiogene) resulting in an in-frame addition of the SuperGloTM(sg)GFP protein to the C-terminus of the expressed protein. Transient transfection of sgGFP-tagged BCMP-11 cDNA into MDA-MB-468 and T-47D cell lines was achieved using SuperfectTM transfection reagent (Qiagen) according to the manufacturers instructions. Transfected cells were washed in phosphate buffered saline (PBS), fixed in 4% paraformaldehyde for 30min, then washed again in PBS before being mounted in an aqueous-based fluorescent mounting medium (Dako Ltd.). Fluorescence images were captured using a DC300F digital camera attached to a DMIRE2 fluorescence microscope (Leica Microsystems (UK) Ltd.)

Transient transfection analysis of a GFP-tagged BCMP-11 cDNA construct demonstrated that the translated GFP-fusion protein was localised to endosome-like organelles in both T-47D and MDA-MB-468 cells. Immunocytochemical analysis of BCMP-11 failed to show any cellular staining even though the antibody reacted with a single band of the correct size by Western blot analysis (data not shown). However, endosomes can be difficult to detect by immunocytochemistry in fixed cultured cells. BCMP-11 has a putative signal sequence and is predicted to be a secreted extracellular protein, consistent with an endosomal location for this protein. In addition, since BCMP-11 was isolated from T-47D membrane preparations (WO 01/63289) it appeared to be bound to the endosomal membrane fraction and/or secreted by the cells and re-bound by a plasma-membrane associated receptor.

Example 2: Immunohistochemical analysis of BCMP-7 and BCMP-11 expression in breast carcinoma

Immunohistochemical analysis was used to determine the expression of BCMP-11 and BCMP-7 in sections of breast carcinoma tissues.

Immunohistochemical analysis was carried out on formalin-fixed paraffin-embedded tissue microarrays containing 1mm sections of breast carcinoma tissue from 50 donors as well as 20 sections of various normal tissues (Clinomics Laboratories Inc., Pittsfield, MA). Slides were deparafinised by two 5min washes in xylene then rehydrated through successive graded ethanol solutions and washed for 5min in PBS. Antigen retrieval was achieved by immersing the slides in 0.01M citrate buffer (pH 6) and microwaving for 10min at full power (950W). The tissue was treated with pepsin (1mg/ml) for 1.5min at room temperature at pH2.

Endogenous hydrogen peroxidase activity was quenched by treating the slides in 3% hydrogen peroxidase/PBS for 10min followed by 2 washes in PBS. The tissue was blocked in 10% donkey serum/PBS for 1hr before addition of 2µg/ml primary polyclonal antibody (in 2.5% donkey serum). The BCMP-11 and BCMP-7 polyclonal antibodies were each raised in rabbits immunised with 2 specific peptides (Abcam Ltd., Cambridge,UK). Peptide sequences were chosen for synthesis based on plots of hydrophobicity, antigenicity, surface probability, and weak homology to other known protein family members. Peptides were synthesised using Fmoc chemistry with a cysteine residue added to the end of each to

enable specific thiol reactive coupling of Keyhole Limpet Haemocyanin prior to immunisation. The BCMP-11 peptides used were; CAQNEEIQEMAQNKFIMLNLMHET (SEQ ID No. 21) and CTYEPRDLPLLIENMKKALRLIQSEL (SEQ ID No. 22), the BCMP-7 peptides used were: VKPGAKKDTKDSRPK (SEQ ID No. 23) and LVYETTDKHLSPDGQ (SEQ ID No. 24). Western blot analysis of T-47D and MDA-MB-468 cell lysates was used to confirm that each antibody cross-reacted with a single band of the predicted size. The oestrogen receptor monoclonal antibody (DAKO M7047) is specific for the N-terminal region of oestrogen receptor alpha.

Following 3 washes in PBS the tissue sections were incubated with biotin conjugated secondary antibodies (Biotin-SP-conjugated AffiniPure Donkey anti-rabbit, Jackson ImmunoResearch) diluted at 1:200 (2.5µg/ml in 2.5% donkey serum/PBS) for 1hr. Slides were washed 3 times in PBS and the tissue incubated with Streptavidin-HRP (Jackson ImmunoResearch) diluted 1:100 (5µg/ml in 2.5% donkey serum/PBS), followed by three 5 min washes in PBS. Antibody signal was detected using DAB substrate solution (Dako Ltd.) according to the manufacturers' instructions.

All sections were screened for the presence of epithelial cells (Moll, R., *et al.*, 1982, Cell 31, 11-24) using an anti-cytokeratin antibody (DAKO, Cat no. M 0821) according to the manufacturers instructions. Those sections without epithelial cells were not used.

Results of immunohistochemical analysis of BCMP-7, BCMP-11, and oestrogen receptor on breast tumour tissue micro-arrays demonstrated that BCMP-7, BCMP-11 and oestrogen receptor protein expression was detected in 48 (83%), 43 (74%) and 35 (59%) of the sections respectively. In each case, expression was restricted to the cancerous epithelial cells of the tumour tissue. BCMP-7 and BCMP-11 staining was predominantly cytoplasmic whereas oestrogen receptor staining was nuclear. Despite the fact that there were some tumour sections that stained for BCMP-7 and BCMP-11 which were not oestrogen receptor positive, overall both BCMP-7 and BCMP-11 protein expression showed a strong correlation with oestrogen receptor. Of the 35 sections that were positive for oestrogen receptor, 34 (97%) were BCMP-7 positive and 32 (91%) were BCMP-11 positive. These data indicate that oestrogen receptor can be involved in the regulation of BCMP-7 and BCMP-11 expression. In support of this, a search of the first 20 Kb of both the BCMP-7 and BCMP-11 promoters has identified 4 and 12 putative oestrogen response elements, respectively. However, since BCMP-7 staining is seen in 16, and BCMP-11 12, oestrogen receptor negative sections, it is clear that factors other than oestrogen receptor can be involved in the regulation of BCMP-7 and BCMP-11 expression. Of the 43 sections that stained positive for BCMP-11, 42 were BCMP-7 positive. This is in agreement with the mRNA expression data for BCMP-7 and BCMP-11 indicating a remarkably concordant expression pattern of both genes in breast cancer tissues (see WO 01/63289 and WO 01/63290).

Example 3: Immunohistochemical analysis of BCMP-7 and BCMP-11 expression in prostate adenocarcinoma

Immunohistochemical analysis was used to determine the expression of BCMP-11 and BCMP-7 in sections of prostate adenocarcinoma tissues.

Immunohistochemical analysis was carried out on formalin-fixed paraffin-embedded tissue microarrays containing 1mm sections of 42 prostate adenocarcinoma and 5 non-malignant sections, as well as 2 normal prostate and 3 benign prostatic hyperplasia sections (BPH) (Clinomics Laboratories Inc., Pittsfield, MA 01201). The protocol used is the same as in Example 2.

Whilst BCMP-7 and BCMP-11 demonstrated a very similar distribution of expression in multiple normal tissues, cell lines, and breast cancer tissues one striking exception was identified, the prostate cancer derived PC3 cell line. Real-time quantitative RT-PCR analysis revealed high levels of BCMP-7 expression in PC3 cells and prostate cancer tissues but almost no detectable BCMP-11 mRNA (WO 01/63290). Immunohistochemical analysis of BCMP-7 and BCMP-11 proteins in multiple prostate cancer donor tissue specimens demonstrated BCMP-7 protein staining in 34 (81%) of the 42 adenocarcinoma sections. In contrast, BCMP-11 staining was detected in only 7 (17%) adenocarcinoma sections. In all instances staining for both BCMP-7 and BCMP-11 was restricted to the cytoplasm of the cancerous epithelial cells.

Example 4: Preparation of a collection of random-primed cDNA fragments

4.A. Collection preparation and transformation in Escherichia coli

4.A.1. Random-primed cDNA fragment preparation and oligo-dT primed cDNA fragment preparation

For mRNA samples isolated from human cells, random-primed or oligo dT-primed cDNA was prepared from 5µg of polyA+ mRNA using a TimeSaver cDNA Synthesis Kit (Amersham Pharmacia Biotech) and with 5µg of random N9-mers or 1µg of oligo dT 18-mers, respectively, according to the manufacturer's instructions. Following phenolic extraction, the cDNA was precipitated and resuspended in water. The resuspended cDNA was phosphorylated by incubating in the presence of T4 DNA Kinase (Biolabs) and ATP for 30min at 37°C. The resulting phosphorylated cDNA was then purified over a separation column (Chromaspin TE 400, Clontech), according to the manufacturers protocol.

4.A.2. Ligation of linkers to blunt-ended cDNA

Oligonucleotide HGX931 (5' end phosphorylated) 1µg/µl and HGX932 1µg/µl were used: sequence of oligo HGX931: 5'-gggccacgaa-3' (SEQ ID No.25); sequence of oligo HGX932: 5'-ttcgtggcccctg-3' (SEQ ID No.26). Linkers were preincubated (5min at 95°C, 10min at 68°C, 15min at 42°C) then cooled down at room temperature and ligated with cDNA fragments at 16°C overnight. Linkers were removed on a separation column (Chromaspin TE 400, Clontech), according to the manufacturers protocol.

4.A.3. Vector preparation

Plasmid pP6 was prepared by replacing the *SpeI/XhoI* fragment of pGAD3S2X (Bartel, P. L., *et al.*, 1993, Using the two-hybrid system to detect protein-protein interactions. In *Cellular Interactions in Development: A Practical Approach*, D. A. Hartley, Ed., Oxford University Press, Oxford; pp 153-179) with the double-stranded oligonucleotide:
5'-ctagccatggccgcaggggccgcggccgcactagtggggatccttaattaaagggccactggggccccggg taccggcgtccccggcgccggcgtgatcacccctaggaattaatttcccggtgaccccgggggagct-3' (SEQ ID No.27)

The pP6 vector was successively digested with *Sfi*1 and *Bam*HI restriction enzymes (Biolabs) for 1hr at 37°C, extracted, precipitated and resuspended in water. Digested plasmid vector backbones were purified on a separation column (Chromaspin TE 400, Clontech), according to the manufacturers protocol.

4.A.4. Ligation between vector and insert of cDNA

The prepared vector was ligated overnight at 15°C with the blunt-ended cDNA described above using T4 DNA ligase (Biolabs). The DNA was then precipitated and resuspended in water.

4.A.5. Library transformation in *Escherichia coli*

The DNA from section 4.A.4 was transformed into Electromax DH10B electrocompetent cells (Gibco BRL) with a Cell Porator apparatus (Gibco BRL). 1ml SOC medium was added and the transformed cells were incubated at 37°C for 1hr. 9ml of SOC medium per tube was added and the cells were plated on LB+ampicillin medium. The colonies were scraped with liquid LB medium, aliquoted and frozen at -80°C.

4.B. Collection transformation in Saccharomyces cerevisiae

The *Saccharomyces cerevisiae* strain (YHGX13 (MATα Gal4Δ Gal80Δ ade2-101::KAN^R, his3, leu2-3, -112, trp1-901, ura3-52 URA3::UASGAL1-LacZ, Met)) was transformed with the cDNA library.

The plasmid DNA contained in *E. coli* were extracted (Qiagen) from aliquoted *E. coli* frozen cells (1.A.5.). *Saccharomyces cerevisiae* yeast YHGX13 in YPGlu were grown.

Yeast transformation was performed according to standard protocol (Giest *et al.* 1995, Yeast, 11, 355-360) using yeast carrier DNA (Clontech). This experiment led to 10^4 to 5 x 10^4 cells/µg DNA. 2 x 10^4 cells were spread on DO-Leu medium per plate. The cells were aliquoted into vials containing 1ml of cells and frozen at -80°C.

4.C. Construction of bait plasmids

For fusions of the bait protein to the DNA-binding domain of the GAL4 protein (for pB35) or Lex A (for pB27) of *S. cerevisiae*, bait fragments were cloned into plasmid pB35 or plasmid pB27.

Plasmid pB35 was prepared by replacing the *Eco*RI/*Xho*I fragment of pCentroMet with PCR fragment *Eco*RI/*Sal*I Gal4MCS of pB6: MCS sequence *Nco*I/*Apa*I:

5' ccatggccggacgggccgcggccgcactagtggggatccttaattaagggccactggggccc 3'(SEQ ID No. 28);

5' gggccccagtggcccttaattaaggatccccactagtgcggccgcggcccgtccggccatgg 3'(SEQ ID NO. 29).

Plasmid pB27 was prepared by replacing the ampicillin resistance of pB20 with the tetracyclin resistance: MCS sequence *Eco*RI/*Pst*I:

5' aattcggggccggacgggccgcggccgcactagtggggatccttaattaagggccactggggcccctcgac ctgca 3'(SEQ ID No. 30)

5' ggtcgaggggccccagtggcccttaattaaggatccccactagtgcggccgcggcccgtccggccccg 3' (SEQ ID No. 31).

The amplification of the bait ORF was obtained by PCR using the Pfu proof-reading *Taq* polymerase (Stratagene), 10pmol of each specific amplification primer and 200ng of plasmid DNA as template.

The PCR program was set up as follows :

94°	45sec	
94°	45sec	x30 cycles
48°	45sec	
72°	6min	
72°	10min	
15°	∞	

The amplification was checked by agarose gel electrophoresis.

The PCR fragments were purified with Qiaquick column (Qiagen) according to the manufacturer's protocol. Purified PCR fragments were digested with adequate restriction enzymes.

The PCR fragments were purified with Qiaquick column (Qiagen) according to the manufacturer's protocol.

The digested PCR fragments were ligated into an adequately digested and dephosphorylated bait vector pB6 according to standard protocol (Sambrook *et al.*) and were transformed into competent bacterial cells. The cells were grown, the DNA extracted and the plasmid was sequenced.

Example 5 : Screening the collection with the two-hybrid in yeast system

5.A. The mating protocol

The mating two-hybrid in yeast system (as described by Legrain *et al.*, 1997, Nature Genetics, 16:277-282) was used but one could also screen the cDNA collection in classical two-hybrid system as described in Fields *et al., supra* or in a yeast reverse two-hybrid system.

The mating procedure allows a direct selection on selective plates because the two fusion proteins are already produced in the parental cells. No replica plating is required.

This protocol was written for the use of the library transformed into the YHGX13 strain.

For bait proteins fused to the DNA-binding domain of GAL4, bait-encoding plasmids were first transformed into *S. cerevisiae* (CG1945 strain (MATaGal4-542 Gal180-538 ade2-101 his3Δ200, leu2-3,112, trp1-901, ura3-52, lys2-801, URA3::GAL4 17mers (X3)-CyC1TATA-LacZ, LYS2::GAL1UAS-GAL1TATA-HIS3 CYH^R)) according to section 4.B. and spread on DO-Trp medium.

For bait proteins fused to the DNA-binding domain of LexA, bait-encoding plasmids were first transformed into *S. cerevisiae* (L40Δgal4 strain (MATa ade2, trp1-901, leu2 3,112, lys2-801, his3Δ200, LYS2::$(lexAop)_4$-HIS3, ura3-52::URA3 $(lexAop)_8$-LacZ, GAL4::Kan^R)) according to section 4.B. and spread on DO-Trp medium.

Day 1, morning : preculture

The cells carrying the bait plasmid obtained at section 4.C. were precultured in 20ml DO-Trp medium and grown at 30°C with vigorous agitation.

Day 1, late afternoon : culture

The OD_{600nm} of the DO-Trp pre-culture of cells carrying the bait plasmid was measured. The OD_{600nm} must lie between 0.1 and 0.5 in order to correspond to a linear measurement.

50ml DO-Trp at OD_{600nm} 0.006/ml was inoculated and grown overnight at 30°C with vigorous agitation.

Day 2 : mating

<u>medium and plates</u>

2 YPGlu 15cm plates
50ml tube with 13ml DO-Leu-Trp-His
100ml flask with 5ml of YPGlu
8 DO-Leu-Trp-His plates
2 DO-Leu-Trp plates

The OD_{600nm} of the DO-Trp culture was measured. It should be around 1.

For the mating, twice as many bait cells as library cells were used. To get a good mating efficiency, one must collect the cells at 10^8 cells per cm^2.

The amount of bait culture (in ml) that makes up 50 OD_{600nm} units for the mating with the prey library was estimated.

A vial containing the library of section 4B was thawed slowly on ice. 1.0ml of the vial was added to 20ml YPGlu. Those cells were recovered at 30°C, under gentle agitation for 10min.

<u>Mating</u>

The 50 OD_{600nm} units of bait culture was placed into a 50ml falcon tube.

The library of section 4B culture was added to the bait culture, then centrifuged, the supernatant discarded and resuspended in 1.6ml YPGlu medium.

The cells were distributed onto two 15cm YPGlu plates with glass beads. The cells were spread by shaking the plates. The plate cells-up at 30°C for 4h30min were incubated.

<u>Collection of mated cells</u>

The plates were washed and rinsed with 6ml and 7ml respectively of DO-Leu-Trp-His. Two parallel serial ten-fold dilutions were performed in 500µl DO-Leu-Trp-His up to 1/10,000. 50µl of each 1/1,000 dilution was spread onto DO-Leu-Trp plates. 22.4ml of collected cells were spread in 400µl aliquots on DO-Leu-Trp-His+Tet plates.

Day 4

Clones that were able to grow on DO-Leu-Trp-His+Tetracyclin were then selected. This medium allows one to isolate diploid clones presenting an interaction.
The His+ colonies were counted on control plates.
The number of His+ cell clones will define which protocol is to be processed :
Upon 60.10^6 Trp+Leu+ colonies :

- if the number His+ cell clones <285: then use the process stamp overlay protocol on all colonies
- if the number of His+ cell clones >285 and <5000: then process via overlay and then stamp overlay protocols on blue colonies (2.B and 2.C).
- if number of His+ cell clones >5000: repeat screen using DO-Leu-Trp-His+Tetracyclin plates containing 3-aminotriazol.

5.B. The X-Gal overlay assay

The X-Gal overlay assay was performed directly on the selective medium plates after scoring the number of His^+ colonies.

Materials

A waterbath was set up. The water temperature should be 50°C.

- 0.5MNa_2HPO_4 pH7.5.
- 1.2% Bacto-agar.
- 2% X-Gal in DMF.
- Overlay mixture : 0.25M Na_2HPO_4 pH7.5, 0.5% agar, 0.1% SDS, 7% DMF (LABOSI), 0.04% X-Gal (ICN). For each plate, 10ml overlay mixture are needed.
- DO-Leu-Trp-His plates.
- Sterile toothpicks.

Experiment

The temperature of the overlay mix should be between 45°C and 50°C. The overlay-mix was poured over the plates in portions of 10ml. When the top layer was settled, they were collected. The plates were incubated overlay-up at 30°C and the time was noted. Blue colonies were checked for regularly. If no blue colony appeared, overnight incubation was performed. Using a pen the number of positives was marked. The positives colonies were streaked on fresh DO-Leu-Trp-His plates with a sterile toothpick.

5.C. The stamp overlay assay

His+ colonies were grown overnight at 30°C in microtiter plates containing DO-Leu-Trp-His+Tetracyclin medium with shaking. The day after the overnight culture, the 96 colonies were stamped on a 15cm plate of DO-Leu-Trp-His. 4 control yeast colonies were spotted on the same plate. After 2 days of growing at 30°C, an overlay assay was

performed on this plate with 80ml of overlay mixture (see section 5.B.). After 2hrs of incubation, the plate was photographed with a CCD camera. The blue intensity was quantified by Genetools® software (SYNGENE) and normalized to the control spots.

Example 6 : Identification of positive clones

6.A. PCR on yeast colonies

Introduction

PCR amplification of fragments of plasmid DNA directly on yeast colonies is a quick and efficient procedure to identify sequences cloned into this plasmid. It is directly derived from a published protocol (Wang H. et al., 1996, Anal. Biochem. 237:145-146). However, it is not a standardized protocol and it varies from strain to strain and it is dependent of experimental conditions (number of cells, *Taq* polymerase source, etc). This protocol should be optimized to specific local conditions.

Materials

- For 1 well, PCR mix composition was:
  - 32.5µl water,
  - 5µl 10X PCR buffer (Pharmacia),
  - 1µl dNTP 10mM,
  - 0.5µl *Taq* polymerase (5U/µl) (Pharmacia),
  - 0.5µl oligonucleotide ABS1 10pmole/µl: 5'-gcgtttggaatcactacagg-3' (SEQ ID No.32)
  - 0.5µl oligonucleotide ABS2 10pmole/µl: 5'-cacgatgcacgttgaagtg-3' (SEQ ID No:33)
- 1N NaOH.

Experiment

The positive colonies were grown overnight at 30°C on a 96-well cell culture cluster (Costar), containing 150µl DO-Leu-Trp-His+Tetracyclin with shaking. The culture was resuspended and 100µl was transferred immediately on a Thermowell 96 (Costar) and centrifuged for 5min at 4,000rpm at room temperature. The supernatant was removed. 5µl NaOH was added to each well and shaken for 1minute. The Thermowell was placed in the thermocycler (GeneAmp 9700, Perkin Elmer) for 5min at 99.9°C and then 10min at 4°C. In each well, the PCR mix was added and shaken well.

The PCR program was set up as followed:

94°C	3minutes	
94°C	30 seconds	
53°C	1 minute 30 seconds	x 35 cycles
72°C	3min	
72°C	5min	
15°C	∞	

The quality, the quantity and the length of the PCR fragment was checked on an agarose gel. The length of the cloned fragment was the estimated length of the PCR fragment minus 300 base pairs that corresponded to the amplified flanking plasmid sequences.

6.B. Plasmids rescue from yeast by electroporation

Introduction

The previous protocol of PCR on yeast cell may not be successful; in such a case, plasmids from yeast by electroporation can be rescued. This experiment allows the recovery of prey plasmids from yeast cells by transformation of *E. coli* with a yeast cellular extract. The prey plasmid can then be amplified and the cloned fragment can be sequenced.

Materials

Plasmid rescue

Glass beads 425-600µm (Sigma)
Phenol/chloroform (1/1) premixed with isoamyl alcohol (Amresco)
Extraction buffer : 2% Triton X100, 1% SDS, 100mM NaCl, 10mM TrisHCl pH8.0, 1mM EDTA pH8.0.
Mix ethanol/NH_4Ac : 6 volumes ethanol with 7.5M NH_4 Acetate, 70% Ethanol and yeast cells in patches on plates.

Electroporation

SOC medium
M9 medium
Selective plates : M9-Leu+Ampicillin
2 mm electroporation cuvettes (Eurogentech)

Experiment

Plasmid rescue

The cell patch on DO-Leu-Trp-His was prepared with the cell culture of section 5.C. The cell of each patch was scraped into an Eppendorf tube, 300µl of glass beads was added in each tube, then, 200µl extraction buffer and 200µl phenol:chloroform:isoamyl alcohol (25:24:1) was added.

The tubes were centrifuged for 10min at 15,000rpm.

180µl supernatant was transferred to a sterile Eppendorf tube and 500µl each of ethanol/NH_4Ac was added and the tubes were vortexed. The tubes were centrifuged for 15min at 15,000rpm at 4°C. The pellet was washed with 200µl 70% ethanol and the ethanol was removed and the pellet was dried. The pellet was resuspended in 10µl water. Extracts were stored at -20°C.

Electroporation

Materials: Electrocompetent MC1066 cells prepared according to standard protocols (Sambrook et al. *supra*).

1µl of yeast plasmid DNA-extract was added to a pre-chilled Eppendorf tube, and kept on ice.

1µl plasmid yeast DNA-extract sample was mixed and 20µl electrocompetent cells was added and transferred in a cold electroporation cuvette.

The Biorad electroporator was set on 200 ohms resistance, 25μF capacity; 2.5 kV. The cuvette was placed in the cuvette holder and electroporation was performed.

1ml of SOC was added into the cuvette and the cell-mix was transferred into a sterile Eppendorf tube. The cells were recovered for 30min at 37°C, then spun down for 1min at 4,000 xg and the supernatant was poured off. About 100μl medium was kept and used to resuspend the cells and spread them on selective plates (*e.g.*, M9-Leu plates). The plates were then incubated for 36hrs at 37°C.

One colony was grown and the plasmids were extracted. The presence and the size of the insert were checked for through enzymatic digestion and agarose gel electrophoresis. The insert was then sequenced.

Example 7 : Protein-protein interaction

For each bait, the previous protocol leads to the identification of prey polynucleotide sequences. Using a suitable software program (*e.g.* Blastwun, available on the Internet site of the University of Washington: http://bioweb.pasteur.fr/seqanal/interfaces/blastwu.html), the mRNA transcript that is encoded by the prey fragment can be identified and whether the fusion protein encoded is in the same open reading frame of translation as the predicted protein or not can be determined.

Alternatively, prey nucleotide sequences can be compared with one another and those which share identity over a significant region (60nt) can be grouped together to form a contiguous sequence (Contig) whose identity can be ascertained in the same manner as for individual prey fragments described above.

Example 8 : Identification of the selected interaction domain

By comparing and selecting the intersection of all isolated fragments that are included in the same polypeptide, one can define the selected interacting domain as illustrated in Figure 1. The selected interacting domain is shown in Table 3.

Example 9 : Major BCMPs Interacting Factors

BCMP-7 and BCMP-11 were concordantly expressed in breast cancers and both showed co-expression with estrogen receptor (Example 2), a known target for breast cancer therapy. Thus, it appeared that there are shared protein binding partners as well as specific protein binding partners. The specific binding partners are expected to occur within a cancer type to determine the different functions of BCMP-7 and BCMP-11, and also between cancer types due to the individual expression of BCMP-7 and BCMP-11 in cancers other than breast cancer.

Both BCMP-11 and BCMP-7 interacted with a clone that represented the human homologue of a rat GPI-anchored metastasis-associated protein (see Tables 2 & 3 and SEQ ID NO:37; GenBank Accession No. NP_055215), consistent with the prediction that BCMP-11 and BCMP-7 were secreted proteins. The rat gene was originally identified as an antigen expressed in a rat pancreatic tumour and was recognised by a monoclonal antibody, C4.4, and thus named C4.4A (Rosel, M., *et al.*, 1998, Oncogene 17:1989-2002).

Constitutively expressing C4.4A tumour cells penetrated through matrigel, a process prevented by C4.4 antibody, indicating a role for C4.4 in metastasis. Expression of human C4.4 in normal tissues was restricted to placenta, skin, oesophagus, and peripheral blood leukocytes, however, multiple tumour tissues had high levels of C4.4 (Wurfel, J., *et al.*, 2001, Gene 262:35-41) Indeed, Seiter et al.(2001, J. Invest. Dermatol. 116:344-347) demonstrated that approximately 50% of carcinoma cell lines, 60% of primary malignant melanomas, and 100% of lymph node metastasis where C4.4A positive. BCMP-11, BCMP-7 and C4.4A were all expressed in carcinoma tissues prone to metastasis. This indicated that BCMP-11 and BCMP-7, which were secreted from tumour cells, associated with C4.4A expressing cells, mediating a signal that resulted in tumour cell invasion and metastasis.

Both BCMP-11 and BCMP-7 interacted with DAG-1 (see Tables 2 & 3 and SEQ ID NO:36; GenBank Accession No. NP_004384). DAG-1 is a transmembrane protein linking the intracellular sarcolemma with the extra-cellular matrix (ECM), binds laminin and is thus also involved in cell-cell adhesion (Ibraghimov-Beskrovnaya, O.; *et al.*, 1992, Nature 355:696-702). Variant isoforms and reduced expression of DAG-1 have been associated with cancer consistent with a loss of cell-ECM interaction leading to increased metastasis (Losasso C. *et al.*, 2000, FEBS Letts. 484(3):194-8; Henry MD. *Et al.* 2001, Hum. Pathol. 32(8):791-5). Thus the interaction of BCMP-7 and BCMP-11 with DAG-1 in cancers indicate a role for this interaction in the adhesion, invasion and growth of cancer cells.

BCMP-7 (and not BCMP-11) interacted specifically with several independent clones encoding the PLAC1 antigen (see Tables 2 & 3 and SEQ ID NO:38; GenBank Accession Nos. AAG22596; NP_068568). The function of PLAC1 is not yet known and it has only limited homology with known proteins. The PLAC1 gene was reported to have a placenta-restricted expression (Cocchia M. *et al.* 2000, Genomics 68:305-312). PLAC1 was predicted to be a secreted protein (Cocchia M et al., supra) consistent with an extracellular interaction with the BCMP-7 protein.

BCMP-11 (and not BCMP-7) interacted with CD68 (see Tables 2 & 3 and SEQ ID NO:39; Swiss-Prot Accession No. P34810), a known 110 kD transmembrane glycoprotein of the heamatopoetic system, associated with the golgi and plasma membrane (Holness, C. L. &; Simmons, D. L. 1993, Blood 81:1607-1613). CD68 belongs to the lysosome associated membrane proteins (LAMPs) family, a group of lysosomal/plasma membrane shuttling proteins (da Silva RP. *Et al.*, 1996, Biochem. Soc. Trans. 24,220-224). Although the function of CD68 is yet largely unknown, the observation that both proteins localized to the same cellular compartments (*i.e.* endosome and plasma membrane since BCMP-11 is a secreted protein) was consistent with a physical interaction between the two proteins. From a functional point of view, it appeared that BCMP-11 secreted by cancer cells modulated the immunological functions of macrophage/monocyte through its interaction with the CD68 antigen.

Example 10: Making of polyclonal and monoclonal antibodies

A BCMP-7 or BCMP-11 protein complex is injected into mice and polyclonal and monoclonal antibodies are made following the procedure set forth in Sambrook et al *supra*.

More specifically, mice are immunized with an immunogen comprising the above mentioned complexes conjugated to keyhole limpet haemocyanin using glutaraldehyde or EDC as is well known in the art. The complexes can also be stabilized by crosslinking as described in WO 00/37483. The immunogen is then mixed with an adjuvant. Each mouse receives four injections of 10µg to 100µg of immunogen, and after the fourth injection, blood samples are taken from the mice to determine if the serum contains antibodies to the immunogen. Serum titer is determined by ELISA or RIA. Mice with sera indicating the presence of antibody to the immunogen are selected for hybridoma production.

Spleens are removed from immune mice and single-cell suspension is prepared (Harlow et al 1988). Cell fusions are performed essentially as described by Kohler et al.. Briefly, P365.3 myeloma cells (ATTC Rockville, Md) or NS-1 myeloma cells are fused with spleen cells using polyethylene glycol as described by Harlow et al (1989). Cells are plated at a density of 2×10^5 cells/well in 96-well tissue culture plates. Individual wells are examined for growth and the supernatants of wells with growth are tested for the presence of complex-specific antibodies by ELISA or RIA using the BCMP-7 or BCMP-11 protein-protein complex as a target protein. Cells in positive wells are expanded and subcloned to establish and confirm monoclonality.

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce sufficient quantities of antibodies for characterization and assay development. Antibodies are tested for binding to the bait BCMP-7 or BCMP-11 polypeptide alone or to the interacting polypeptide alone, to determine which are specific for the BCMP-7 or BCMP-11 protein complex as opposed to those that bind to the individual proteins.

Monoclonal antibodies against each of the BCMP-7 or BCMP-11 protein complexes are prepared in a similar manner by mixing specified proteins together, immunizing an animal, fusing spleen cells with myeloma cells and isolating clones which produce antibodies specific for the protein complex, but not for individual proteins.

Example 11: Modulating compounds identification

Each specific BCMP-7 or BCMP-11 protein complex is used to screen for modulating compounds.

One appropriate construction for this modulating compound screening can be:

- bait polynucleotide inserted in pB35 or pB27;
- prey polynucleotide inserted in pP6;
- transformation of these two vectors in a permeable yeast cell;
- growth of the transformed yeast cell on a medium containing compound to be tested,
- and observation of the growth of the yeast cells.

The following results obtained from these Examples, as well as the teachings in the specification are set forth in the Tables below.

Preferred features of each embodiment of the invention are as for each of the other embodiments *mutatis mutandis.* All publications, including but not limited to patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

While the invention has been described in terms of the various preferred embodiments, the skilled artisan will appreciate that various modifications, substitutions, omissions and changes may be made without departing from the scope thereof. Accordingly, it is intended that the present invention be limited by the scope of the following claims, including equivalents thereof.

Table 1 : bait name and sequence

Bait name	Bait nucleic acid sequence	Nucleic Positions	Bait amino acid sequence	Bait construction
BCMP-11	ataaaaaaggaaaagaggcctcctcagacactctcaagaggatggggagat gacatcacttgggtacaaacttatgaagaaggtctcttttatgctcaaaaaagtaa gaagccattaatggttattcatcacctggaggattgtcaatactctcaagcactaa agaaagtatttgcccaaaatgaagaaatacaagaaatggctcagaataagttc gtcatgctaaaccttatgcatgaaaccactgataagaatttatcacctgatgggc aatatgtgcctagaatcatgtttgtagacccttctttaacagttagagctgacatag ctggaagatactctaacagattgtacacatatgagcctcgggatttacccctattg atagaaaacatgaagaaagcattaagacttattcagtcagagcta **(SEQ ID NO:1)**	[70-498]	IKKEKRPPQTLSRGW GDDITWVQTYEEGLF YAQKSKKPLMVIHHLE DCQYSQALKKVFAQN EEIQEMAQNKFVMLN LMHETTDKNLSPDGQ YVPRIMFVDPSLTVRA DIAGRYSNRLYTYEP RDLPLLIENMKKALRLI QSEL **(SEQ ID NO:3)**	pB35
BCMP-7	agagataccacagtcaaacctggagccaaaaaggacacaaaggactctcg acccaaactgccccagaccctctccagaggttggggtgaccaactcatctgga ctcagacatatgaagaagctctatataaatccaagacaagcaacaaacccttg atgattattcatcacttggatgagtgcccacacagtcaagctttaaagaaagtgttt gctgaaaataaagaaatccagaaattggcagagcagtttgtcctcctcaatctg gtttatgaaacaactgacaaacacctttctcctgatggccagtatgtccccaggat tatgtttgttgacccatctctgacagttagagccgatatcactggaagatattcaaa ccgtctctatgcttacgaacctgcagatacagctctgttgcttgacaacatgaaga aagctctcaagttgctgaagactgaattg **(SEQ ID NO:2)**	[61-525]	RDTTVKPGAKKDTKD SRPKLPQTLSRGWG DQLIWTQTYEEALYK SKTSNKPLMIIHHLDE CPHSQALKKVFAENK EIQKLAEQFVLLNLVY ETTDKHLSPDGQYVP RIMFVDPSLTVRADIT GRYSNRLYAYEPADT ALLLDNMKKALKLLKT EL **(SEQ ID NO:4)**	pB27

Table 2: bait-prey interactions

Bait name	Interacting polypeptide identified	Prey construction
BCMP-7	DAG-1; Accession Nos. AAA81779; NP_004384 **(SEQ ID NO:36)**	Human Placenta Random Primed 2
BCMP-11	DAG-1; Accession Nos. AAA81779; NP_004384 **(SEQ ID NO:36)**	Human Placenta Random Primed 2
BCMP-7	C4.4A; Accession Nos. AAD13751; NP_055215 **(SEQ ID NO:37)**	Human Placenta Random Primed 2
BCMP-7	C4.4A; Accession Nos. AAD13751; NP_055215 **(SEQ ID NO:37)**	Human placenta oligo dT primed 1
BCMP-11	C4.4A; Accession Nos. AAD13751; NP_055215 **(SEQ ID NO:37)**	Human Placenta Random Primed 2
BCMP-7	PLAC1; Accession Nos. AAG22596; NP_068568 **(SEQ ID NO:38)**	Human Placenta Random Primed 2
BCMP-7	PLAC1; Accession Nos. AAG22596; NP_068568 **(SEQ ID NO:38)**	Human placenta oligo dT primed 1
BCMP-11	CD68; Accession No. NP_001242 **(SEQ ID NO:39)**	Human placenta oligo dT primed 1
BCMP-7	DAG-1; Accession No. AAA81779 **(SEQ ID NO:36)**	Human Breast Epithelial cells RP1

Table 3: Selected Interaction Domain

Bait protein name	Interacting polypeptide name	Selected interaction domain nucleic acid sequence	Selected interaction domain amino acid sequence	Prey construction
BCMP-7	DAG-1	cacaaccaccaccaagaagccacgagtatccacaccaa aaccagcaacgccttcaactgactccaccaccaccacga ctcgcaggccaaccaagaaaccacggacaccccggcc agtgccccgggtcaccaccaaagtttccatcaccagattg gaaactgcctcaccgcctactcgtattcgcaccaccacca gtggagtgccccgtggcggagaacccaaccagcgccca gagctcaagaaccatattgacagggtagatgcctgggttg gcacctactttgaggtgaagatcccgtcagacactttctatg accatgaggacaccaccactgacaagctgaagctgacc ctgaaactgcgggagcagcagctggtgggcgagaagtc ctgggtacagttcaacagcaacagccagc **(SEQ ID NO:5)**	TTTTKKPRVSTPKPATPSTDSTTTTT RRPTKKPRTPRPVPRVTTKVSITRL ETASPPTRIRTTTSGVPRGGEPNQR PELKNHIDRVDAWVGTYFEVKIPSD TFYDHEDTTTDKLKLTLKLREQQLV GEKSWVQFNSNSQ **(SEQ ID NO:13)**	Human Placenta random primed 2
BCMP-11	DAG-1	cacaaccaccaccaagaagccacgagtatccacaccaa aaccagcaacgccttcaactgactccaccaccaccacga ctcgcaggccaaccaagaaaccacggacaccccggcc agtgccccgggtcaccaccaaagtttccatcaccagattg gaaactgcctcaccgcctactcgtattcgcaccaccacca gtggagtgccccgtggcggagaacccaaccagcgccca gagctcaagaaccatattgacagggtagatgcctgggttg gcacctactttgaggtgaagatcccgtcagacactttctatg accatgaggacaccaccactgacaagctgaagctgacc ctgaaactgcgggagcagcagctggtgggcgagaagtc ctgggtacagttcaacagcaacagccagctcatgtatgg **(SEQ ID NO:6)**	TTTTKKPRVSTPKPATPSTDSTTTTT RRPTKKPRTPRPVPRVTTKVSITRL ETASPPTRIRTTTSGVPRGGEPNQR PELKNHIDRVDAWVGTYFEVKIPSD TFYDHEDTTTDKLKLTLKLREQQLV GEKSWVQFNSNSQLMY **(SEQ ID NO:14)**	Human Placenta random primed 2
BCMP-7	C4.4A	cgtggacgtctgcaccgaggccgtgggggcggtggaga ccatccacggacaattctcgctggcagtgcsgggttgcggt tcgggactccccggcaagaatgaccgcggcctggatcttc acgggcttctggcgttcatccagctgcagcaatgcgctcag gatcgctgcaacgccaagctcaacctcacctcgcgggcg ctcgacccggcaggtaatgagagtgcatacccgcccaac	VDVCTEAVGAVETIHGQFSLAVXGC GSGLPGKNDRGLDLHGLLAFIQLQQ CAQDRCNAKLNLTSRALDPAGNES AYPPNGVECYSCVGLSREACQGTS PPVVSCYNASDHVYKGCFDGNVTL TAANVTVSLPVRGCVQDEFCTRDG	Human Placenta random primed 2

		ggcgtggagtgctacagctgtgtgggcctgagccgggag gcgtgccagggtacatcgccgccggtcgtgagctgctaca acgccagcgatcatgtctacaagggctgcttcgacggcaa cgtcaccttgacggcagctaatgtgactgtgtccttgcctgtc cggggctgtgtccaggatgaattctgcactcgggatggagt aacaggcccagggttcacgctcagtggctcctgttgccag gggtcccgctgtaactctgacctccgcaacaagacctactt ctcccctcgaatcccacccttgtccggctgccccctccag agcccacgactgtggcctcaaccacatctgtcaccacttct acctcggccccagtgagacccacatccaccaccaaacc catgccagcgccaaccagtcagactccgagacagggag tagaacacgaggcctcccgggatgaggagcccaggttg actggaggcgccgctggccaccaggaccgcagcaattc agggcagtatcctgcaaaaggggggccccagcagcccc ataataaaggctgtgtggctcccacagctggattggcagc ccttctgttggccgtggctgctggtgtcctactgtga **(SEQ ID NO:7)**	VTGPGFTLSGSCCQGSRCNSDLRN KTYFSPRIPPLVRLPPPEPTTVASTT SVTTSTSAPVRPTST **(SEQ ID NO:15)**	
BCMP-7	C4.4A	aacaggcccagggttcacgctcagtggctcctgttgccag gggtcccgctgtaactctgacctccgcaacaagacctactt ctcccctcgaatcccacccttgtccggctgccccctccag agcccacgactgtggcctcaaccacatctgtcaccacttct acctcggccccagtgagacccacatccaccaccaaacc catgccagcgccaaccagtcagactccgagacagggag tagaacacgaggcctcccgggatgaggagcccaggttg actggaggcgccgctggccaccaggaccgcagcaattc agggcagtatcctgcaaaaggggggccccagcagcccc ataataaaggctgtgtggctcccacagctggattggcagc ccttctgttggccgtggctgctggtgtcctactgtga **(SEQ ID NO:8)**	TGPGFTLSGSCCQGSRCNSDLRNK TYFSPRIPPLVRLPPPEPTTVASTTS VTTSTSAPVRPTSTTKPMPAPTSQT PRQGVEHEASRDEEPRLTGGAAGH QDRSNSGQYPAKGGPQQPHNKGC VAPTAGLAALLLAVAAGVLL***(SEQ ID NO:16)**	Human Placenta oligo dT primed 1
BCMP-11	C4.4A	gcsgggttgcggttcgggactccccggcaagaatgaccg cggcctggatcttcacgggcttctggcgttcatccagctgca gcaatgcgctcaggatcgctgcaacgccaagctcaacct cacctcgcgggcgctcgacccggcaggtaatgagagtgc ataccgcccaacggcgtggagtgctacagctgtgtgggc ctgagccgggaggcgtgccagggtacatcgccgccggtc gtgagctgctacaacgccagcgatcatgtctacaagggct gcttcgacggcaacgtcaccttgacggcagctaatgtgact gtgtccttgcctgtccggggctgtgtccaggatgaattctgca ctcgggatggagtaacaggcccagggttcacgctcagtg gctcctgttgccaggggtcccgctgtaactctgacctccgca acaagacctacttctcccctcgaatcccacccttgtccgg	XGCGSGLPGKNDRGLDLHGLLAFIQ *LQQCAQDRCNAKLNLTSRALDPAG* NESAYPPNGVECYSCVGLSREACQ GTSPPVVSCYNASDHVYKGCFDGN VTLTAANVTVSLPVRGCVQDEFCTR DGVTGPGFTLSGSCCQGSRCNSDL RNKTYFSPRIPPLVRLPPPEPTTVAS TTSVTTSTSAPVRPTST **(SEQ ID NO:17)**	Human Placenta *random primed 2*

		ctgccccctccagagcccacgactgtggcctcaaccacat ctgtcaccacttctacctcggccccagtgagacccacatcc accac **(SEQ ID NO:9)**		
BCMP-7	PLAC1	catggtcacagtgcacccttcatgctaaacaacgatgtgt gtgtacactttcatgaactacacttgggcctgggttgccccc caaaccatgttcagccacacgcctaccagttcacctaccgt gttactgaatgtggcatcagggccaaagctgtctctcagga catggttatctacagcactgagatacactactcttctaaggg cacgccatctaagtttgtgatcccagtgtcatgtgctgcccc ccaaaagtccccatggctcaccaagccctgctccatgaga gtagccagcaagagcagggccacagcccagaaggatg agaaatgctacgaggtgttcagcttgtcacagtccagtcaa aggcccaactgcgattgtccaccttgtgtcttcagtgaagaa gagcataccca **(SEQ ID NO:10)**	MVTVHPFMLNNDVCVHFHELHLGL GCPPNHVQPHAYQFTYRVTECGIR AKAVSQDMVIYSTEIHYSSKGTPSK FVIPVSCAAPQKSPWLTKPCSMRVA SKSRATAQKDEKCYEVFSLSQSSQ RPNCDCPPCVFSEEEHT **(SEQ ID NO:18)**	Human Placenta random primed 2
BCMP-7	PLAC1	cacctaccgtgttactgaatgtggcatcagggccaaagctg tctctcaggacatggttatctacagcactgagatacactact cttctaagggcacgccatctaagtttgtgatcccagtgtcatg tgctgccccccaaaagtccccatggctcaccaagccctgc tccatgagagtagccagcaagagcagggccacagccca gaaggatgagaaatgctacgaggtgttcagcttgtcacagt ccagtcaaaggcccaactgcgattgtccaccttgtgtcttca gtgaagaagagcatacccaggtcccttgtcaccaagcag gggctcaggaggctcaacctctgcagccatctcactttcttg atatttctgaggattggtctcttcacacagatgatatgattggg tccatgtga **(SEQ ID NO:11)**	TYRVTECGIRAKAVSQDMVIYSTEIH YSSKGTPSKFVIPVSCAAPQKSPWL TKPCSMRVASKSRATAQKDEKCYE VFSLSQSSQRPNCDCPPCVFSEEE HTQVPCHQAGAQEAQPLQPSHFLD ISEDWSLHTDDMIGSM***(SEQ ID NO:19)**	Human Placenta oligo dT primed 1
BCMP-11	CD68	caccactcacaggacaaccaccacaggcaccaccagc cacggacccacgactgccactcacaaccccaccaccac cagccatggaaacgtcacagttcatccaacaagcaatag cactgccaccagccagggaccctcaactgccactcacag tcctgccaccactagtcatggaaatgccacggttcatccaa caagcaacagcactgccaccagcccaggattcaccagtt ctgcccacccagaaccacctccaccctctccgagtcctag cccaacctccaaggagaccattggagactacacgtggac caatggttcccagccctgtgtccacctccaagcccagattc	TTHRTTTTGTTSHGPTTATHNPTTT SHGNVTVHPTSNSTATSQGPSTAT HSPATTSHGNATVHPTSNSTATSPG FTSSAHPEPPPPSPSPSPTSKETIG DYTWTNGSQPCVHLQAQIQIRVMY TTQGGGEAWGISVLNPNKTKVQGS CEGAHPHLLLSFPYGHLSFGFMQDL QQKVVYLSYMAVEYNVSFPHAAKW TFSAQNASLRDLQAPLGQSFSCSN	Human Placenta oligo dT primed 1

	agattcgagtcatgtacacaacccagggtggaggagagg cctgggcatctctgtactgaaccccacaaaaccaaggt ccagggaagctgtgagggtgcccatcccacctgcttctct cattcccctatggacacctcagcttggattcatgcaggacc tccagcagaaggttgtctacctgagctacatggcggtgga gtacaatgtgtccttccccacgcagcaaagtggacattct cggctcagaatgcatcccttcgagatctccaagcacccctg gggcagagcttcagttgcagcaactcgagcatcattctttca ccagctgtccacctcgacctgctctccctgaggctccaggc tgctcagctgcccacacagggggtctttgggcaaagtttctc ctgccccagtgaccggtccatcttgctgcctctcatcatcgg cctgatccttcttggcctcctcgccctggtgcttattgcttctgtgc atcatccggagacgcccatcgcctaccaggccctctga **(SEQ ID NO:12)**	SSIILSPAVHLDLLSLRLQAAQLPHT GVFGQSFSCPSDRSILLPLIIGLILLG LLALVLIAFCIIRRRPSAYQAL*(**SEQ ID NO:20)**	
BCMP-7 DAG-1	agctggcaccacagttcctggccagattcgcccaacgatg accattcctggctatgtggagcctactgcagttgctaccct cccacaaccaccaccaagaagccacgagtatccacacc aaaaccagcaacgccttcaactgactccaccaccaccac gactcgcaggccaaccaagaaaccacggacaccccgg ccagtgccccgggtcaccaccaaagtttccatcaccagatt ggaaactgcctcaccgcctactcgtattcgcaccaccacc agtggagtgccccgtggcggagaacccaaccagcgccc agagctcaagaaccatattgacagggtagatgcctgggtt ggcacctactttgaggtgaagatccccgtcagacacttctat gaccatgaggacaccaccactgacaagctgaagctgac cctgaaactgcgggagcagcagctggtgggcgagaagt cctgggtacagttcaacagcaacagccagctcatgtatgg ccttccgacagca **(SEQ ID NO:34)**	AGTTVPGQIRPTMTIPGYVEPTAVA TPPTTTTKKPRVSTPKPATPSTDST TTTTRRPTKKPRTPRPVPRVTTKVSI TRLETASPPTRIRTTTSGVPRGGEP NQRPELKNHIDRVDAWVGTYFEVKI PSDTFYDHEDTTTDKLKLTLKLREQ QLVGEKSWVQFNSNSQLMYGLPDS **(SEQ ID NO:35)**	Human Breast Epithelial cells random primed 1

CLAIMS

1. An isolated or recombinant protein complex comprising:
   (a) an interacting polypeptide which:
      (i) comprises or consists of the amino acid sequence of SEQ ID NOS:14, 17 or 20; or
      (ii) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence of SEQ ID NOS: 14, 17 or 20, which retains the interaction domain of the interacting polypeptide;
   and a BCMP-11 polypeptide which:
      (i) comprises or consists of the amino acid sequence of SEQ ID NO:1; or
      (ii) is a derivative having one or more amino acid substitutions, modifications, deletions or insertions relative to the amino acid sequence of SEQ ID NO: 1 which retains the ability to interact with the interacting polypeptide;
   or
   (b) an interacting polypeptide which:
      (i) comprises or consists of the amino acid sequence of SEQ ID NOS:13, 15, 16, 18, 19 or 35; or
      (ii) is a derivative having one or more amino acid substitutions, deletions or insertions relative to the amino acid sequence of SEQ ID NOS:13, 15, 16, 18, 19 or 35, which retains the interaction domain of the interacting polypeptide;
   and a BCMP-7 polypeptide which:
      (i) comprises or consists of the amino acid sequence of SEQ ID NO:2; or
      (ii) is a derivative having one or more amino acid substitutions, modifications, deletions or insertions relative to the amino acid sequence of SEQ ID NO:2 which retains the ability to interact with the interacting polypeptide.

2. A protein complex as defined in claim 1, wherein the interacting polypeptide of part (a) comprises or consists of the amino acid sequence of SEQ ID NOS:36, 37 or 39, and the interacting polypeptide of part (b) comprises or consists of the amino acid sequence of SEQ ID NOS:36, 37 or 38.

3. A method of producing a protein complex as defined in claim 1 or 2, comprising:
   (a) providing an expression system comprising a nucleic acid capable of producing a BCMP-7 polypeptide as defined in claim 1(a) or a BCMP-11 polypeptide as defined in claim 1(b), wherein said expression system is present in a compatible host cell;
   (b) providing an expression system comprising a nucleic acid capable of producing an interacting polypeptide as defined in claim 1, or claim 2, wherein said expression system is present in a compatible host cell;

wherein, under appropriate culture conditions, said host cells produce polypeptides which on combination form the protein complex.

4. The method of claim 3, wherein the expression systems of (a) and (b), are present within the same host cell.

5. A recombinant host cell comprising the expression systems of claim 4.

6. An antibody that specifically recognises a protein complex as defined in claim 1 or 2, wherein the antibody does not recognise a BCMP-7 polypeptide as defined in claim 1(b), or a BCMP-11 polypeptide as defined in claim 1(a), or an interacting polypeptide as defined in claim 1 or 2, when said BCMP-7 or BCMP-11 or the interacting polypeptide is in an uncomplexed form.

7. An antibody as defined in claim 6, which is polyclonal, monoclonal, bispecific, chimeric or humanised or is conjugated to a therapeutic moiety, detectable label, second antibody or a fragment thereof, a cytotoxic agent or cytokine.

8. A method of modulating the activity of a BCMP-7 polypeptide as defined in claim 1(b), or a BCMP-11 polypeptide as defined in claim 1(a), comprising contacting said polypeptide with an interacting polypeptide as defined in claim 1 or 2, or an antibody as defined in claim 6 or 7.

9. A method of modulating the activity of an interacting polypeptide as defined in claim 1 or 2, comprising contacting said polypeptide with a BCMP-7 as defined in claim 1(b), or a BCMP-11 polypeptide as defined in claim 1(a), or an antibody as defined in claim 6 or 7.

10. A method of screening for/or identifying an agent that modulates the interaction of a BCMP-7 polypeptide as defined in claim 1(b) or a BCMP-11 polypeptide as defined in claim 1(a), with an interacting polypeptide as defined in claim 1 or 2, comprising:

(a) contacting a protein complex comprising the BCMP-7 polypeptide or the BCMP-11 polypeptide and the interacting polypeptide with a candidate agent; and
(b) determining whether the agent modulates the interaction.

11. A method according to claim 10, comprising:

(a) contacting a protein complex as defined in claim 1 or 2, with a candidate agent;
(b) contacting a protein complex as defined in claim 1 or 2, with a control agent; and
(c) determining whether the candidate agent modulates the interaction by comparing the amount of complex present in part (a) with that present in part (b).

12. The method of claim 11, wherein the amount of complex present is determined using an antibody as defined in claim 6 or 7.

13. An agent identified using the method of any one of claims 10 to 12, which modulates the interaction of a BCMP-7 polypeptide as defined in claim 1(b) or a BCMP-11 polypeptide as defined in claim 1(a), with an interacting polypeptide as defined in claim 1 or 2.

14. The use of an agent which modulates the interaction of a BCMP-7 polypeptide as defined in claim 1(b) or a BCMP-11 polypeptide as defined in claim 1(a), with an interacting polypeptide as defined in claim 1 or 2, or an antibody as defined in claim 6 or 7, as a medicament.

15. A pharmaceutical composition comprising an antibody as defined in claim 6 or 7.

16. A pharmaceutical composition comprising an agent which modulates the interaction of a BCMP-7 polypeptide as defined in claim 1(b) or a BCMP-11 polypeptide as defined in claim 1(a), with an interacting polypeptide as defined in claim 1 or 2.

17. The use of an agent which modulates the interaction of a BCMP-7 polypeptide as defined in claim 1(b) or a BCMP-11 polypeptide as defined in claim 1(a), with an interacting polypeptide as defined in claim 1 or 2, or an antibody as defined in claim 6 or 7, in the manufacture of a medicament for the treatment or prophylaxis of cancer.

18. A method of screening for, and/or diagnosis of cancer in a subject, and/or monitoring the effectiveness of a cancer therapy, comprising measuring the level or activity of a protein complex as defined in claim 1 or 2, in a biological sample obtained from said subject.

19. The method as defined in claim 18, wherein the level or activity is additionally compared to a control sample or a previously determined reference range.

20. The method as defined in claim 18 or 19, wherein the level or activity of the complex is determined using an antibody as defined in claim 6 or 7.

21. A diagnostic kit adapted to detect a protein complex as defined in claim 1 or 2, comprising an antibody as defined in claim 6 or 7, and instructions for use.

FIGURE 1